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(54) Title: 3, BB-CIS-DIHYDROTETRABENAZINE FOR THE TREATMENT OF A PROLIFERATIVE DISEASE OR AN IN-
FLAMMATION

(57) Abstract: The invention provides the use of a compound for the manufacture of a medicament for the prophylaxis or treatment
of a proliferative disease or an inflammatory disease, the compound being 3,11b-cis-dihydrotetrabenazine or a pharmaceutically
acceptable salt thereof.



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3, 11B-CIS-DIHYDROTETRABENAZINE FOR THE TREATMENT OF A PROLIFERATIVE DISEASE OR AN INFLAMMATION

This invention relates to the use of dihydrotetrabenazine in the prophylaxis or treatment of inflammatory diseases and cancers.

Background of the Invention

- 5 Cancer is the collective term given to a group of diseases characterised by abnormal and uncontrolled cell growth. Normally, cells grow and divide to form new cells only when the body needs them. When cells grow old and die, new cells take their place. Mutations in the genes within a cell can sometimes disrupt this process such that new cells form when the body does not need them, and old cells do not die
- 10 when they should. The extra cells form a mass of tissue, called a growth, neoplasm, or tumour. Tumours can be either benign (not cancerous) or malignant (cancerous). Benign tumors do not spread to other parts of the body, and they are rarely a threat to life whereas malignant tumors can spread (metastasize) and may be life threatening. Cancers originate within a single cell and hence can be classified by the
- 15 type of cell in which they originate and by the location of the cell. Thus, Adenomas originate from glandular tissue, Carcinomas originate in epithelial cells, Leukaemias start in the bone marrow stem cells, Lymphomas originate in lymphatic tissue, Melanomas arise in melanocytes, Sarcomas begin in the connective tissue of bone or muscle and Teratomas begin within germ cells.
- 20 Various methods exist for treating cancers and the commonest are surgery, chemotherapy and radiation therapy. In general the choice of therapy will depend upon the location and grade of the tumour and the stage of the disease. If the tumour is localized, surgery is often the preferred treatment. Examples of common surgical procedures include prostatectomy for prostate cancer and mastectomy for
- 25 breast cancer. The goal of the surgery can be either the removal of only the tumour, or the entire organ. Since a single cancer cell can grow into a sizeable tumor, removing only the tumour leads to a greater chance of recurrence. Chemotherapy involves the treatment of cancer with drugs that can destroy or prevent the growth of cancer cells. Alternative mechanisms exploited by cancer chemotherapies
- 30 include anti-angiogenic agents which act to disrupt the blood vessels supplying the tumour and immunotherapeutic agents which act to enhance the host immune

response against the tumour tissue. Normal cells grow and die in a controlled way. When cancer occurs, cells in the body that are not normal keep dividing and forming more cells without control. One class of anticancer drugs acts by killing dividing cells or by stopping them from growing or multiplying. Healthy cells can also be harmed, especially those that divide quickly, and this can lead to side effects. Radiation therapy involves the use of ionizing radiation to kill cancer cells and shrink tumours. Radiation therapy injures or destroys cells in the area being treated (the "target tissue") by damaging their genetic material, making it impossible for these cells to continue to grow and divide. Although radiation damages both cancer cells and normal cells, most normal cells can recover from the effects of radiation and function properly. The goal of radiation therapy is to damage as many cancer cells as possible, while limiting harm to nearby healthy tissue. Radiation therapy may be used to treat almost every type of solid tumor, including cancers of the brain, breast, cervix, larynx, lung, pancreas, prostate, skin, spine, stomach, uterus, or soft tissue sarcomas. Radiation can also be used to treat leukaemia and lymphoma (cancers of the blood-forming cells and lymphatic system, respectively).

It has been reported that ligands of the sigma (σ) receptors have an ability to inhibit the growth of tumour cells (Berthois *et al.*, *British Journal of Cancer*, (2003), 88, 438-446). Bourrie *et al.* (*Current Opinion in Investigational Drugs*, (2004) 5(11):1158-63) have also reported that the two sigma receptor subtypes and their two related proteins are also expressed on tumor cells.

Sigma receptors are cell surface receptors that were once considered to be a subtype of opiate receptors but it has now been demonstrated, following characterization of the receptor and the discovery of specific ligands for the receptors, that they are distinct from opiate receptors (see Bourrie *et al.*, (2004)).

Sigma receptors can be classified into the sigma-1 (σ -1) and sigma-2 (σ -2) subtypes. The σ -1 receptor, which is believed to contain 223 amino acids, has been cloned (Hanner *et al.*, *Proc. Natl. Acad. Sci. USA*, (1996) 93:8072-8077) and it has been found to exhibit no primary sequence homology to any other receptor class. However, it does have a 30.3% identity to the sequence of a fungal sterol C8-C7

isomerase and has also been found to be related to another protein of unknown function, SRBP2 (SR-31747 binding-protein 2), which shares a high homology with this enzyme. The σ -2 receptor has not yet been cloned.

Wang *et al.*, *Breast Cancer Research & Treatment*, (2004), 87(3):205-14, examined
5 the expression of the sigma-1 receptor in human breast cancer and found over-expression of sigma-1 receptor mRNA in 64% of breast cancers compared to normal tissue from which they concluded that some normal and most neoplastic breast epithelial cells and cell lines commonly express the sigma-1 receptor. They also found that high concentrations of haloperidol, a non-specific sigma-1 ligand,
10 inhibited the growth of these cells and potentiated the effect of chemotherapy *in vitro*.

Berthois *et al.*, (2003) described studies in which the effect of a sigma receptor ligand SR31747A on the proliferation of human epithelial breast and prostate cancer cell lines was measured. They reported that, *in vitro*, nanomolar
15 concentrations of SR31747A dramatically inhibited cell proliferation in both hormone-responsive and hormone-unresponsive cancer cell lines. They also found that tumour development was significantly decreased in mice that had been treated with SR31747A.

Spruce *et al.*, *Cancer Research*, (2004), 64: 4875-4886, have reported that small
20 molecule σ -1 receptor antagonists inhibit the growth of evolving and established hormone-sensitive and hormone-insensitive mammary carcinoma xenographs, orthotopic prostate tumours and p53-null lung carcinoma xenographs in immunocompromised mice in the absence of side effects. They found that the σ -1 antagonists induced apoptosis (programmed cell death) in tumour cells but not in
25 most normal cell types. Spruce *et al.* concluded that the use of σ -1 antagonists could conceivably offer a way of killing tumours while sparing normal tissues.

Other reports of the use of σ -1 receptor ligands in inhibiting the proliferation of cancer cells can be found in the references cited in Spruce *et al.* (2004), see for example Brent *et al.*, *Eur. J. Pharmacol.*, (1995), 278:151-60 and Crawford *et al.*,
30 *Cancer Research*, (2002), 62:313-22.

It is therefore fairly well established that sigma receptor ligands can inhibit proliferation of cancer cell lines and can induce apoptosis in cancer cells and, on the basis of this evidence, it is envisaged that sigma receptor ligands such as sigma-1 antagonists, will prove useful in the treatment of cancer.

- 5 Sigma receptor ligands have also been reported as having anti-inflammatory activity. For example, Bourrie *et al.*, *Eur. J. Pharmacol.*, (2002), 456(1-3):123-31 report that the Sanofi-Synthelabo Recherche compound SSR125329A (chemical name [(Z)-3-(4-adamantan-2-yl-3,5-dichloro-phenyl)-allyl]-cyclohexyl-ethylamine) is a high affinity sigma receptor ligand with potent anti-inflammatory properties.
- 10 Bourrie *et al.*, conclude that the results of their studies provide substantial evidence that sigma receptor ligands may represent a new effective approach for rheumatoid arthritis treatment.

Another Sanofi sigma receptor antagonist (SR31747) is currently in clinical trials for the treatment of rheumatoid arthritis.

- 15 Tetrabenazine (Chemical name: 1, 3, 4,6,7,11b-hexahydro-9,10-dimethoxy-3-(2-methylpropyl)-2H-benzo(a)quinolizin-2-one) has been in use as a pharmaceutical drug since the late 1950s. Initially developed as an anti-psychotic, tetrabenazine is currently used in the symptomatic treatment of hyperkinetic movement disorders such as Huntington's disease, hemiballismus, senile chorea, tic, tardive dyskinesia
- 20 and Tourette's syndrome, see for example Jankovic *et al.*, *Am. J. Psychiatry.* (1999) Aug; 156(8):1279-81 and Jankovic *et al.*, *Neurology* (1997) Feb; 48(2):358-62.

The chemical structure of tetrabenazine is as shown in Figure 1 below.

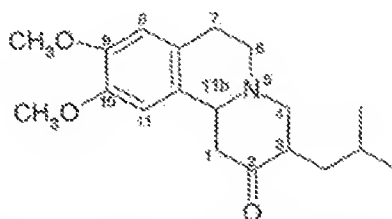


Figure 1- Structure of tetrabenazine

- 25 The compound has chiral centres at the 3 and 11b carbon atoms and hence can, theoretically, exist in a total of four isomeric forms, as shown in Figure 2.

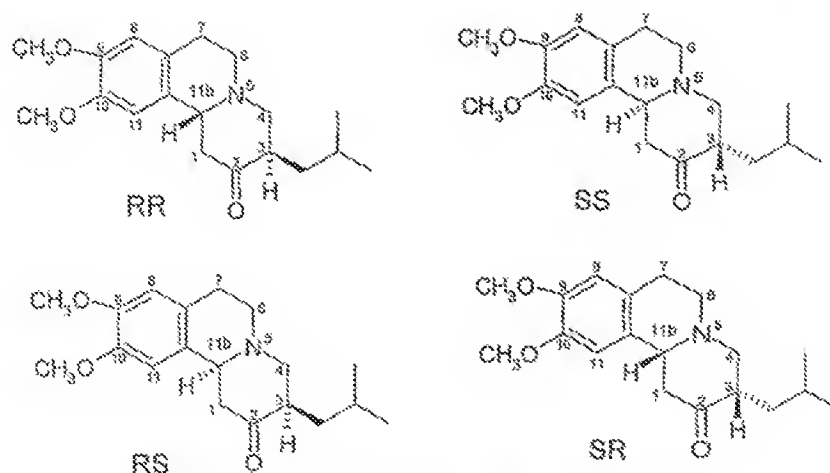


Figure 2 – Possible tetraabenazine isomers

In Figure 2, the stereochemistry of each isomer is defined using the “R and S” nomenclature developed by Cahn, Ingold and Prelog, see *Advanced Organic Chemistry* by Jerry March, 4th Edition, John Wiley & Sons, New York, 1992, pages 109-114. In Figure 2 and elsewhere in this patent application, the designations “R” or “S” are given in the order of the position numbers of the carbon atoms. Thus, for example, *RS* is a shorthand notation for 3*R*,11*bS*. Similarly, when three chiral centres are present, as in the dihydrotetraabenazines described below, the designations “R” or “S” are listed in the order of the carbon atoms 2, 3 and 11*b*. Thus, the 2*S*,3*R*,11*bR* isomer is referred to in short hand form as *SRR* and so on.

Commercially available tetraabenazine is a racemic mixture of the *RR* and *SS* isomers and it would appear that the *RR* and *SS* isomers (hereinafter referred to individually or collectively as *trans*-tetraabenazine because the hydrogen atoms at the 3 and 11*b* positions have a *trans* relative orientation) are the most thermodynamically stable isomers.

Tetraabenazine has somewhat poor and variable bioavailability. It is extensively metabolised by first-pass metabolism, and little or no unchanged tetraabenazine is typically detected in the urine. The major metabolite is dihydrotetraabenazine (Chemical name: 2-hydroxy-3-(2-methylpropyl)-1,3,4,6,7,11*b*-hexahydro-9,10-dimethoxy-benzo(a)quinolizine) which is formed by reduction of the 2-keto group in tetraabenazine, and is believed to be primarily responsible for the activity of the

drug (see Mehvar *et al.*, *Drug Metab. Disp.*, 15, 250-255 (1987) and *J. Pharm. Sci.*, 76, No.6, 461-465 (1987)).

Four dihydrotetrabenazine isomers have previously been identified and characterised, all of them being derived from the more stable *RR* and *SS* isomers of the parent tetrabenazine and having a *trans* relative orientation between the hydrogen atoms at the 3 and 11b positions) (see Kilbourn *et al.*, *Chirality*, 9:59-62 (1997) and Brossi *et al.*, *Helv. Chim. Acta.*, vol. XLI, No. 193, pp1793-1806 (1958). The four isomers are (+)- α -dihydrotetrabenazine, (-)- α -dihydrotetrabenazine, (+)- β -dihydrotetrabenazine and (-)- β -dihydrotetrabenazine. The structures of the four known dihydrotetrabenazine isomers are considered to be as shown in Figure 3.

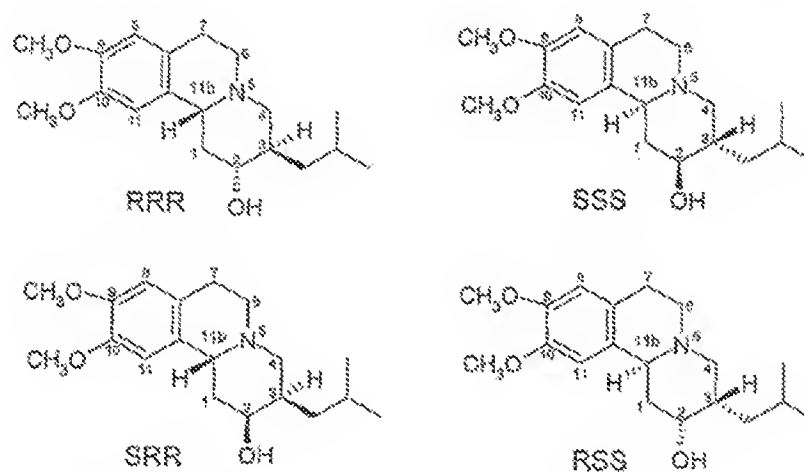


Figure 3 – Structures of known isomers of dihydrotetrabenazine

Kilbourn *et al.* (see *Eur. J. Pharmacol.*, 278:249-252 (1995) and *Med. Chem. Res.*, 5:113-126 (1994)) investigated the specific binding of individual radio-labelled dihydrotetrabenazine isomers in the conscious rat brain. They found that the (+)- α -¹¹C]dihydrotetrabenazine (2*R*,3*R*,11*bR*) isomer accumulated in regions of the brain associated with higher concentrations of the neuronal membrane dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT2). However, the essentially inactive (-)- α -¹¹C]dihydrotetrabenazine isomer was almost uniformly distributed in the brain, suggesting that specific binding to DAT and VMAT2 was not occurring. The *in vivo* studies correlated with *in vitro* studies which demonstrated that the (+)- α -¹¹C]dihydrotetrabenazine isomer exhibits a K_i

for [³H]methoxytetrabenazine >2000-fold higher than the K_i for the (-)-α-
[¹¹C]dihydrotetrabenazine isomer.

Our earlier International patent application No. PCT/GB2005/000464 discloses the
preparation and use of pharmaceutical dihydrotetrabenazine isomers derived from
5 the unstable *RS* and *SR* isomers (hereinafter referred to individually or collectively
as *cis*-tetrabenazine because the hydrogen atoms at the 3 and 11b positions have a
cis relative orientation) of tetrabenazine. PCT/GB2005/000464 contains
experimental data showing that *cis*-dihydrotetrabenazine isomers bind to sigma-1
and sigma-2 receptors but does not disclose any therapeutic uses making use of the
10 sigma receptor binding activity.

Summary of the Invention

The present invention relates to the use of the *cis*-dihydrotetrabenazine described in
our earlier application no. PCT/GB2005/000464 in the treatment of inflammatory
diseases and cancers.

15 Accordingly, in a first aspect, the invention provides 3, 11b-*cis*-
dihydrotetrabenazine for use in the prophylaxis or treatment of a proliferative
disease or an inflammatory disease.

In one embodiment, the proliferative disease is a cancer.

Accordingly, the invention provides 3, 11b-*cis*-dihydrotetrabenazine for use in the
20 prophylaxis or treatment of a proliferative disease such as a cancer.

The invention also provides:

- The use of 3, 11b-*cis*-dihydrotetrabenazine for the manufacture of a
medicament for the treatment of a proliferative disease such as a cancer.
- The use of *cis*-dihydrotetrabenazine for the manufacture of a medicament
25 for the prophylaxis or treatment of a disease state or condition (e.g. a
cancer) arising from abnormal cell growth

- A method for treating a disease or condition (e.g. a cancer) comprising or arising from abnormal cell growth in a mammal, the method comprising administering to the mammal a therapeutically effective amount of *cis*-dihydrotetrabenazine.
- 5 • A method for treating a disease or condition (e.g. a cancer) comprising or arising from abnormal cell growth in a mammal, which method comprises administering to the mammal *cis*-dihydrotetrabenazine in an amount effective in inhibiting abnormal cell growth.
- 10 • A method for alleviating or reducing the incidence of a disease or condition (e.g. a cancer) comprising or arising from abnormal cell growth in a mammal, which method comprises administering to the mammal *cis*-dihydrotetrabenazine in an amount effective in inhibiting abnormal cell growth.

It is envisaged that the compounds of the invention will be useful in the treatment or prophylaxis of any one more cancers selected from:

15 adenomas;
 carcinomas;
 leukaemias;
 lymphomas;
20 melanomas;
 sarcomas; and
 teratomas.

Particular examples of cancers which may be inhibited or treated include, but are not limited to, a carcinoma, for example a carcinoma of the bladder, breast, colon

25 (e.g. colorectal carcinomas such as colon adenocarcinoma and colon adenoma),
 kidney, epidermal, liver, lung, for example adenocarcinoma, small cell lung cancer and non-small cell lung carcinomas, oesophagus, gall bladder, ovary, pancreas e.g. exocrine pancreatic carcinoma, stomach, cervix, thyroid, prostate, or skin, for example squamous cell carcinoma; a hematopoietic tumour of lymphoid lineage,

30 for example leukaemia, acute lymphocytic leukaemia, B-cell lymphoma, T-cell

lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell lymphoma, or Burkett's lymphoma; a hematopoietic tumour of myeloid lineage, for example acute and chronic myelogenous leukaemias, myelodysplastic syndrome, or promyelocytic leukaemia; thyroid follicular cancer; a tumour of mesenchymal
5 origin, for example fibrosarcoma or rhabdomyosarcoma; a tumour of the central or peripheral nervous system, for example astrocytoma, neuroblastoma, glioma or schwannoma; melanoma; seminoma; teratocarcinoma; osteosarcoma; xenoderma pigmentosum; keratoctanthoma; thyroid follicular cancer; or Kaposi's sarcoma.

More particularly, the cancers that may be treated or inhibited by the use of *cis*-
10 dihydrotetrabenazine are those cancers that are sensitive to sigma receptor ligands, for example σ -1 antagonists.

Further examples of cancers that may be treated or inhibited by the use of *cis*-dihydrotetrabenazine are those cancers in which sigma receptors are over-expressed.

15 In one particular embodiment, the cancer is a mammary carcinoma.

In another particular embodiment, the cancer is a prostate tumour, for example an orthotopic prostate tumour.

In a further particular embodiment, the cancer is a lung cancer.

In another general embodiment, the invention provides 3, 11b-*cis*-
20 dihydrotetrabenazine for use in the treatment of an inflammatory disease.

The invention also provides:

- The use of 3, 11b-*cis*-dihydrotetrabenazine for the manufacture of a medicament for the prophylaxis or treatment of an inflammatory disease.
- A method for the prophylaxis or treatment of an inflammatory disease or
25 condition in a patient (e.g. a mammal such as a human), which method comprises administering to the mammal a therapeutically effective amount of 3,11b-*cis*-dihydrotetrabenazine.

Examples of inflammatory diseases and conditions include, but are not limited to, rheumatoid arthritis, osteoarthritis, traumatic arthritis, gouty arthritis, rubella arthritis, psoriatic arthritis, and other arthritic conditions; acute or chronic inflammatory disease states such as the inflammatory reaction induced by
5 endotoxin or inflammatory bowel disease; Reiter's syndrome, gout, rheumatoid spondylitis, chronic pulmonary inflammatory disease, Crohn's disease and ulcerative colitis.

Particular inflammatory diseases and conditions are those that are sensitive to sigma receptor ligands, for example, sigma receptor antagonists.

10 One particular inflammatory disease is rheumatoid arthritis.

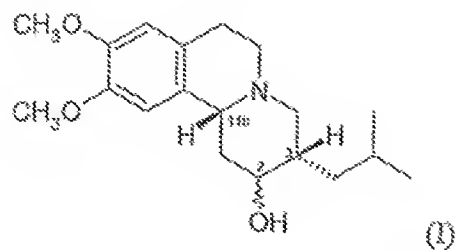
The *cis*-dihydrotetrabenazine used in the present invention is 3,11b, *cis*-dihydrotetrabenazine.

The 3,11b-*cis*-dihydrotetrabenazine used in the invention may be in substantially pure form, for example at an isomeric purity of greater than 90%, typically greater
15 than 95% and more preferably greater than 98%.

The term "isomeric purity" in the present context refers to the amount of 3,11b-*cis*-dihydrotetrabenazine present relative to the total amount or concentration of dihydrotetrabenazine of all isomeric forms. For example, if 90% of the total dihydrotetrabenazine present in the composition is 3,11b-*cis*-dihydrotetrabenazine,
20 then the isomeric purity is 90%.

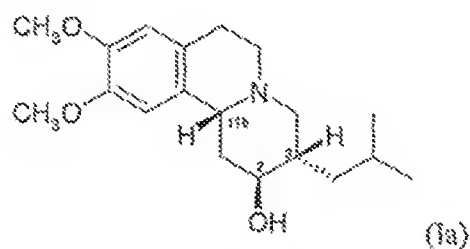
The 11b-*cis*-dihydrotetrabenazine used in the invention may be in the form of a composition which is substantially free of 3,11b-*trans*-dihydrotetrabenazine, preferably containing less than 5% of 3,11b-*trans*-dihydrotetrabenazine, more preferably less than 3% of 3,11b-*trans*-dihydrotetrabenazine, and most preferably
25 less than 1% of 3,11b-*trans*-dihydrotetrabenazine.

The term "3,11b-*cis*-" as used herein means that the hydrogen atoms at the 3- and 11b-positions of the dihydrotetrabenazine structure are in the *cis* relative orientation. The isomers of the invention are therefore compounds of the formula (I) and antipodes (mirror images) thereof.

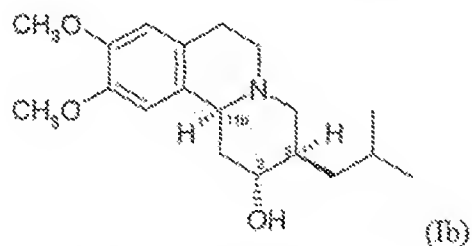


There are four possible isomers of dihydrotetrabenazine having the 3,11b-*cis* configuration and these are the 2*S*,3*S*,11b*R* isomer, the 2*R*,3*R*,11b*S* isomer, the 2*R*,3*S*,11b*R* isomer and the 2*S*,3*R*,11b*S* isomer. The four isomers have been isolated and characterised and, in another aspect, the invention provides individual isomers of 3,11b-*cis*-dihydrotetrabenazine. In particular, the invention provides:

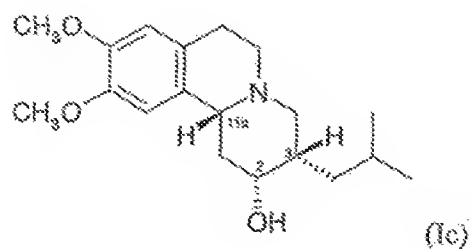
(a) the 2*S*,3*S*,11b*R* isomer of 3,11b-*cis*-dihydrotetrabenazine having the formula (Ia):



(b) the 2*R*,3*R*,11b*S* isomer of 3,11b-*cis*-dihydrotetrabenazine having the formula (Ib):

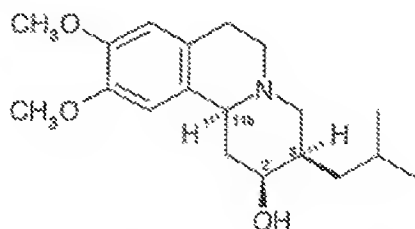


(c) the 2*R*,3*S*,11b*R* isomer of 3,11b-*cis*-dihydrotetrabenazine having the formula (Ic):



and

(d) the 2*S*,3*R*,11*bS* isomer of 3,11*b*-*cis*-dihydrotetrabenzazine having the formula (Id):



- 5 The individual isomers of the invention can be characterised by their spectroscopic, optical and chromatographic properties, and also by their absolute stereochemical configurations as determined by X-ray crystallography.

Without implying any particular absolute configuration or stereochemistry, the four novel isomers may be characterised as follows:

10 Isomer A

Optical activity as measured by ORD (methanol, 21°C): laevorotatory (-)

IR Spectrum (KBr solid), ¹H-NMR spectrum (CDCl₃) and ¹³C-NMR spectrum (CDCl₃) substantially as described in Table 1.

Isomer B

- 15 Optical activity as measured by ORD (methanol, 21°C): dextrorotatory (+)

IR Spectrum (KBr solid), ¹H-NMR spectrum (CDCl₃) and ¹³C-NMR spectrum (CDCl₃) substantially as described in Table 1, and X-ray crystallographic properties as described in Example 4.

Isomer C

- 20 Optical activity as measured by ORD (methanol, 21°C): dextrorotatory (+)

IR Spectrum (KBr solid), ¹H-NMR spectrum (CDCl₃) and ¹³C-NMR spectrum (CDCl₃) substantially as described in Table 2.

Isomer D

Optical activity as measured by ORD (methanol, 21°C): laevorotatory (-)

IR Spectrum (KBr solid), ^1H -NMR spectrum (CDCl_3) and ^{13}C -NMR spectrum (CDCl_3) substantially as described in Table 2.

ORD values for each isomer are given in the examples below but it is noted that such values are given by way of example and may vary according to the degree of
5 purity of the isomer and the influence of other variables such as temperature fluctuations and the effects of residual solvent molecules.

The enantiomers A, B, C and D may each be presented in a substantially enantiomerically pure form or as mixtures with other enantiomers of the invention.

The terms "enantiomeric purity" and "enantiomerically pure" in the present context
10 refer to the amount of a given enantiomer of 3,11b-*cis*-dihydrotetrabenazine present relative to the total amount or concentration of dihydrotetrabenazine of all enantiomeric and isomeric forms. For example, if 90% of the total dihydrotetrabenazine present in the composition is in the form of a single enantiomer, then the enantiomeric purity is 90%.

15 By way of example, in each aspect and embodiment of the invention, each individual enantiomer selected from Isomers A, B, C and D may be present in an enantiomeric purity of at least 55% (e.g. at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5% or 100%).

The isomers of the invention may also be presented in the form of mixtures of one
20 or more of Isomers A, B, C and D. Such mixtures may be racemic mixtures or non-racemic mixtures. Examples of racemic mixtures include the racemic mixture of Isomer A and Isomer B and the racemic mixture of Isomer C and Isomer D.

Pharmaceutically Acceptable Salts

Unless the context requires otherwise, a reference in this application to
25 dihydrotetrabenazine and its isomers, includes within its scope not only the free base of the dihydrotetrabenazine but also its salts, and in particular acid addition salts.

Particular acids from which the acid addition salts are formed include acids having a pKa value of less than 3.5 and more usually less than 3. For example, the acid

addition salts can be formed from an acid having a pKa in the range from +3.5 to -3.5.

Preferred acid addition salts include those formed with sulphonic acids such as methanesulphonic acid, ethanesulphonic acid, benzene sulphonic acid, toluene
5 sulphonic acid, camphor sulphonic acid and naphthalene sulphonic acid.

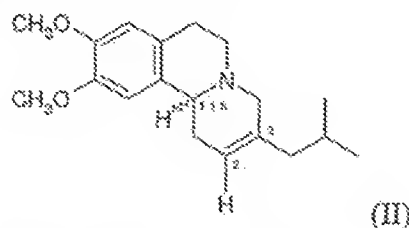
One particular acid from which acid addition salts may be formed is methanesulphonic acid.

Acid addition salts can be prepared by the methods described herein or conventional chemical methods such as the methods described in *Pharmaceutical*
10 *Salts: Properties, Selection, and Use*, P. Heinrich Stahl (Editor), Camille G. Wermuth (Editor), ISBN: 3-90639-026-8, Hardcover, 388 pages, August 2002. Generally, such salts can be prepared by reacting the free base form of the compound with the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media such as ether, ethyl acetate,
15 ethanol, isopropanol, or acetonitrile are used.

The salts are typically pharmaceutically acceptable salts. However, salts that are not pharmaceutically acceptable may also be prepared as intermediate forms which may then be converted into pharmaceutically acceptable salts. Such non-pharmaceutically acceptable salt forms also form part of the invention.

20 Methods for the Preparation of Dihydrotetrabenazine Isomers

The dihydrotetrabenazine of the invention can be prepared by a process comprising the reaction of a compound of the formula (II):



with a reagent or reagents suitable for hydrating the 2,3-double bond in the
25 compound of formula (II) and thereafter where required separating and isolating a desired dihydrotetrabenazine isomer form.

The hydration of the 2,3-double bond can be carried out by hydroboration using a borane reagent such as diborane or a borane-ether (e.g. borane-tetrahydrofuran (THF)) to give an intermediate alkyl borane adduct followed by oxidation of the alkyl borane adduct and hydrolysis in the presence of a base. The hydroboration is typically carried out in a dry polar non-protic solvent such as an ether (e.g. THF), usually at a non-elevated temperature, for example room temperature. The borane-alkene adduct is typically oxidised with an oxidising agent such as hydrogen peroxide in the presence of a base providing a source of hydroxide ions, such as ammonium hydroxide or an alkali metal hydroxide, e.g. potassium hydroxide or sodium hydroxide. The hydroboration-oxidation-hydrolysis sequence of reactions of Process A typically provides dihydrotetrabenazine isomers in which the hydrogen atoms at the 2- and 3-positions have a *trans* relative orientation.

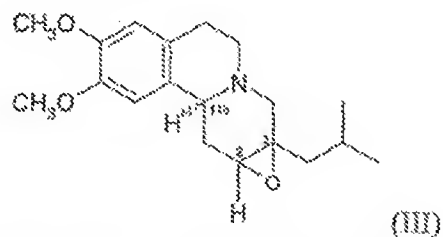
Compounds of the formula (II) can be prepared by reduction of tetrabenazine to give a dihydrotetrabenazine followed by dehydration of the dihydrotetrabenazine. Reduction of the tetrabenazine can be accomplished using an aluminium hydride reagent such as lithium aluminium hydride, or a borohydride reagent such as sodium borohydride, potassium borohydride or a borohydride derivative, for example an alkyl borohydride such as lithium tri-*sec*-butyl borohydride. Alternatively, the reduction step can be effected using catalytic hydrogenation, for example over a Raney nickel or platinum oxide catalyst. Suitable conditions for performing the reduction step are described in more detail below or can be found in US 2,843,591 (Hoffmann- La Roche) and Brossi *et al.*, *Helv. Chim. Acta.*, vol. XLI, No. 193, pp1793-1806 (1958).

Because the tetrabenazine used as the starting material for the reduction reaction is typically a mixture of the *RR* and *SS* isomers (i.e. *trans*-tetrabenazine), the dihydrotetrabenazine formed by the reduction step will have the same *trans* configuration about the 3- and 11b positions and will take the form of one or more of the known dihydrotetrabenazine isomers shown in Figure 3 above. Thus Process A may involve taking the known isomers of dihydrotetrabenazine, dehydrating them to form the alkene (II) and then "rehydrating" the alkene (II) using conditions that give the required novel *cis* dihydrotetrabenazine isomers of the invention.

Dehydration of the dihydrotetrabenazine to the alkene (II) can be carried out using a variety of standard conditions for dehydrating alcohols to form alkenes, see for example J. March (*idem*) pages 389-390 and references therein. Examples of such conditions include the use of phosphorus-based dehydrating agents such as phosphorus halides or phosphorus oxyhalides, e.g. POCl_3 and PCl_5 . As an alternative to direct dehydration, the hydroxyl group of the dihydrotetrabenazine can be converted to a leaving group L such as halogen (e.g. chlorine or bromine) and then subjected to conditions (e.g. the presence of a base) for eliminating H-L. Conversion of the hydroxyl group to a halide can be achieved using methods well known to the skilled chemist, for example by reaction with carbon tetrachloride or carbon tetrabromide in the presence of a trialkyl or triaryl phosphine such as triphenyl phosphine or tributyl phosphine.

The tetraabenazine used as the starting material for the reduction to give the dihydrotetraabenazine can be obtained commercially or can be synthesised by the method described in US 2,830,993 (Hoffmann-La Roche).

Another process (Process B) for preparing a dihydrotetrabenazine of the invention comprises subjecting a compound of the formula (III):



to conditions for ring-opening the 2,3-epoxide group in the compound of the
20 formula (III), and thereafter where required separating and isolating a desired
dihydrotetrabenazine isomer form.

The ring-opening can be effected in accordance with known methods for epoxide ring openings. However, a currently preferred method of ring-opening the epoxide is reductive ring opening which can be achieved using a reducing agent such as borane-THF. Reaction with borane-THF can be carried out in a polar non-protic solvent such as ether (e.g. tetrahydrofuran) usually at ambient temperature, the borane complex thus formed being subsequently hydrolysed by heating in the

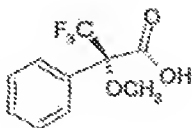
presence of water and a base at the reflux temperature of the solvent. Process B typically gives rise to dihydrotetrabenazine isomers in which the hydrogen atoms at the 2- and 3-positions have a *cis* relative orientation.

5 The epoxide compounds of the formula (III) can be prepared by epoxidation of an alkene of the formula (II) above. The epoxidation reaction can be carried out using conditions and reagents well known to the skilled chemist, see for example J. March (*idem*), pages 826-829 and references therein. Typically, a per-acid such as *meta*-chloroperbenzoic acid (MCPBA), or a mixture of a per-acid and a further oxidising agent such as perchloric acid, may be used to bring about epoxidation.

10 When the starting materials for processes A and B above are mixtures of enantiomers, then the products of the processes will typically be pairs of enantiomers, for example racemic mixtures, possibly together with diastereoisomeric impurities. Unwanted diastereoisomers can be removed by techniques such as chromatography (e.g. HPLC) and the individual enantiomers can
15 be separated by a variety of methods known to the skilled chemist. For example, they can be separated by means of:

- (i) chiral chromatography (chromatography on a chiral support); or
- (ii) forming a salt with an optically pure chiral acid, separating the salts of the two diastereoisomers by fractional crystallisation and then releasing the
20 dihydrotetrabenazine from the salt; or
- (iii) forming a derivative (such as an ester) with an optically pure chiral derivatising agent (e.g. esterifying agent), separating the resulting epimers (e.g. by chromatography) and then converting the derivative to the dihydrotetrabenazine.

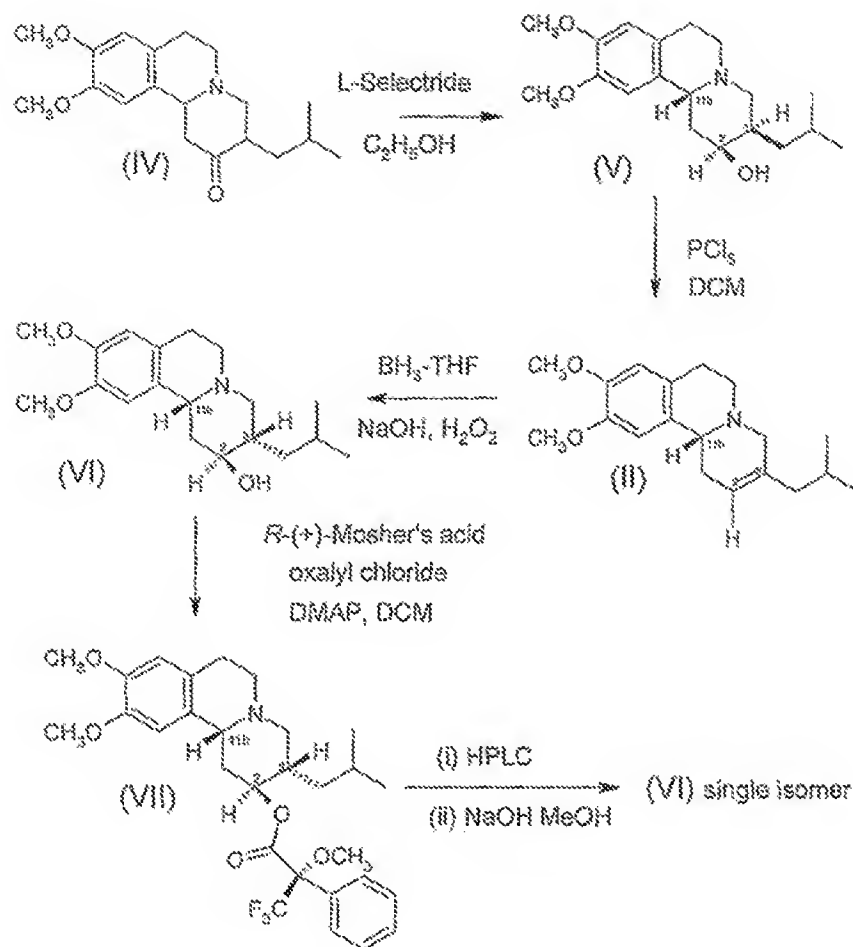
One method of separating pairs of enantiomers obtained from each of Processes A
25 and B, and which has been found to be particularly effective, is to esterify the hydroxyl group of the dihydrotetrabenazine with an optically active form of Mosher's acid, such as the *R* (+) isomer shown below, or an active form thereof:



The resulting esters of the two enantiomers of the dihydrobenzazine can then be separated by chromatography (e.g. HPLC) and the separated esters hydrolysed to give the individual dihydrobenzazine isomers using a base such as an alkali metal hydroxide (e.g. NaOH) in a polar solvent such as methanol.

- 5 As an alternative to using mixtures of enantiomers as the starting materials in processes A and B and then carrying out separation of enantiomers subsequently, processes A and B can each be carried out on single enantiomer starting materials leading to products in which a single enantiomer predominates. Single enantiomers of the alkene (II) can be prepared by subjecting RR/SS tetrabenazine to a
- 10 stereoselective reduction using lithium tri-*sec*-butyl borohydride to give a mixture of SRR and RSS enantiomers of dihydrotetrabenazine, separating the enantiomers (e.g. by fractional crystallisation) and then dehydrating a separated single enantiomer of dihydrotetrabenazine to give predominantly or exclusively a single enantiomer of the compound of formula (II).
- 15 Processes A and B are illustrated in more detail below in Schemes 1 and 2 respectively.

Scheme 1



Scheme 1 illustrates the preparation of individual dihydrotetrabenazine isomers having the $2S,3S,11bR$ and $2R,3R,11bS$ configurations in which the hydrogen atoms attached to the 2- and 3-positions are arranged in a *trans* relative orientation. This reaction scheme includes Process A defined above.

The starting point for the sequence of reactions in Scheme 1 is commercially available tetrabenazine (IV) which is a racemic mixture of the *RR* and *SS* optical isomers of tetrabenazine. In each of the *RR* and *SS* isomers, the hydrogen atoms at the 3- and 11*b*-positions are arranged in a *trans* relative orientation. As an alternative to using the commercially available compound, tetrabenazine can be synthesised according to the procedure described in US patent number 2,830,993 (see in particular example 11).

The racemic mixture of RR and SS tetrabenazine is reduced using the borohydride reducing agent lithium tri-*sec*-butyl borohydride ("L-Selectride") to give a mixture of the known 2*S*,3*R*,11*bR* and 2*R*,3*S*,11*bS* isomers (V) of dihydrotetrabenazine, of which only the 2*S*,3*R*,11*bR* isomer is shown for simplicity. By using the more
5 sterically demanding L-Selectride as the borohydride reducing agent rather than sodium borohydride, formation of the RRR and SSS isomers of dihydro-tetrabenazine is minimised or suppressed.

The dihydrotetrabenazine isomers (V) are reacted with a dehydrating agent such as phosphorus pentachloride in a non-protic solvent such as a chlorinated hydrocarbon
10 (for example chloroform or dichloromethane, preferably dichloromethane) to form the unsaturated compound (II) as a pair of enantiomers, only the *R*-enantiomer of which is shown in the Scheme. The dehydration reaction is typically carried out at a temperature lower than room temperature, for example at around 0-5°C.

The unsaturated compound (II) is then subjected to a stereoselective re-hydration to
15 generate the dihydrotetrabenazine (VI) and its mirror image or antipode (not shown) in which the hydrogen atoms at the 3- and 11*b*-positions are arranged in a *cis* relative orientation and the hydrogen atoms at the 2- and 3-positions are arranged in a *trans* relative orientation. The stereoselective rehydration is accomplished by a hydroboration procedure using borane-THF in tetrahydrofuran
20 (THF) to form an intermediate borane complex (not shown) which is then oxidised with hydrogen peroxide in the presence of a base such as sodium hydroxide.

An initial purification step may then be carried out (e.g. by HPLC) to give the product (V) of the rehydration reaction sequence as a mixture of the 2*S*,3*S*,11*bR* and 2*R*,3*R*,11*bS* isomers of which only the 2*S*,3*S*,11*bR* isomer is shown in the
25 Scheme. In order to separate the isomers, the mixture is treated with *R* (+) Mosher's acid, in the presence of oxalyl chloride and dimethylaminopyridine (DMAP) in dichloromethane to give a pair of diastereoisomeric esters (VII) (of which only one diastereoisomer is shown) which can then be separated using HPLC. The individual esters can then be hydrolysed using an alkali metal
30 hydroxide such as sodium hydroxide to give a single isomer (VI).

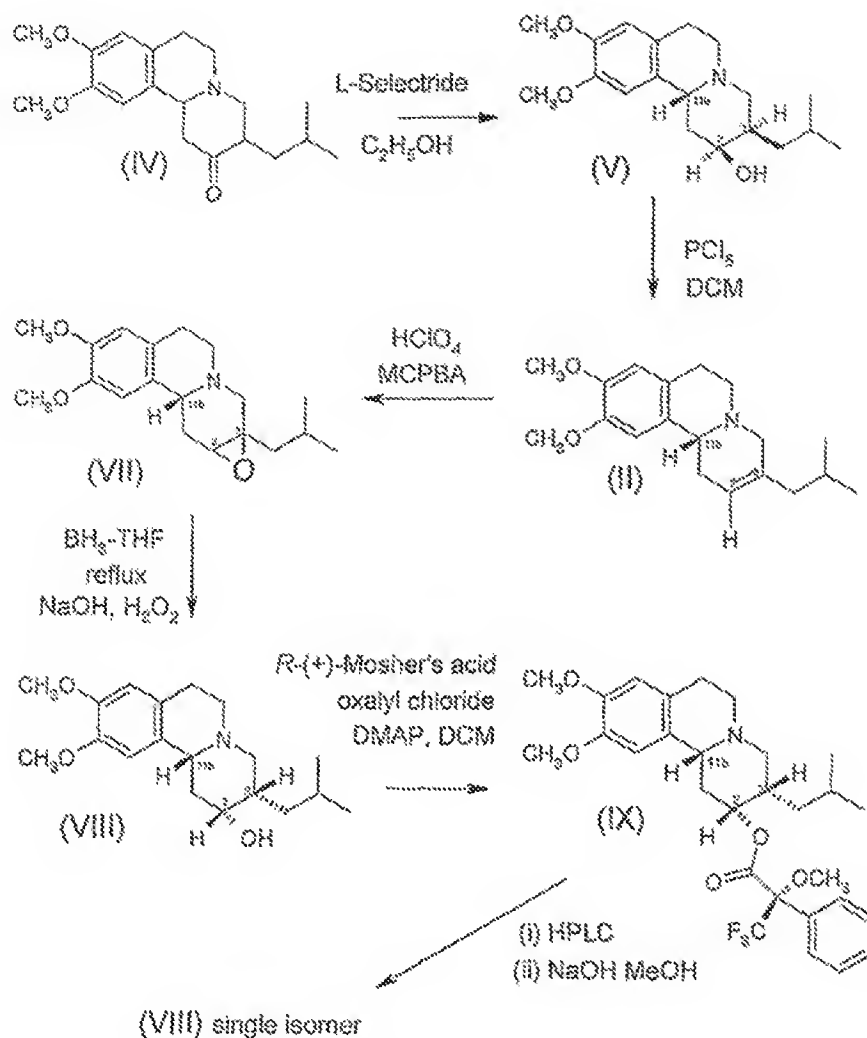
In a variation of the sequence of steps shown in Scheme 1, following the reduction of RR/SS tetrabenazine, the resulting mixture of enantiomers of the dihydrotetrabenazine (V) can be separated to give the individual enantiomers.

- 5 Separation can be carried out by forming a salt with a chiral acid such as (+) or (-) camphorsulphonic acid, separating the resulting diastereoisomers by fractional crystallisation to give a salt of a single enantiomer and then releasing the free base from the salt.

- The separated dihydrotetrabenazine enantiomer can be dehydrated to give a single enantiomer of the alkene (II). Subsequent rehydration of the alkene (II) will then
10 give predominantly or exclusively a single enantiomer of the cis-dihydrotetrabenazine (VI). An advantage of this variation is that it does not involve the formation of Mosher's acid esters and therefore avoids the chromatographic separation typically used to separate Mosher's acid esters.

- Scheme 2 illustrates the preparation of individual dihydrotetrabenazine isomers
15 having the 2*R*,3*S*,11*bR* and 2*S*,3*R*,11*bS* configurations in which the hydrogen atoms attached to the 2- and 3-positions are arranged in a *cis* relative orientation. This reaction scheme includes Process B defined above.

Scheme 2



In Scheme 2, the unsaturated compound (II) is produced by reducing tetrabenazine to give the 2S,3R,11bR and 2R,3S,11bS isomers (V) of dihydrotetrabenazine and dehydrating with PCl_5 in the manner described above in Scheme 1. However, instead of subjecting the compound (II) to hydroboration, the 2,3-double bond is converted to an epoxide by reaction with *meta*-chloroperbenzoic acid (MCPBA) and perchloric acid. The epoxidation reaction is conveniently carried out in an alcohol solvent such as methanol, typically at around room temperature.

The epoxide (VII) is then subjected to a reductive ring opening using borane-THF as an electrophilic reducing agent to give an intermediate borane complex (not shown) which is then oxidised and cleaved with hydrogen peroxide in the presence

of an alkali such as sodium hydroxide to give a dihydrotetrabenazine (VIII) as a mixture of the 2*R*,3*S*,11*bR* and 2*S*,3*R*,11*bS* isomers, of which only the 2*R*,3*S*,11*bR* is shown for simplicity. Treatment of the mixture of isomers (VIII) with *R* (+) Mosher's acid in the presence of oxalyl chloride and dimethylaminopyridine (DMAP) in dichloromethane gives a pair of epimeric esters (IX) (of which only one epimer is shown) which can then be separated by chromatography and hydrolysed with sodium hydroxide in methanol in the manner described above in relation to Scheme 1.

Biological Activity

10 The *cis*-dihydrotetrabenazine compounds of the invention bind to both sigma-1 and sigma-2 receptors, as illustrated in the examples below. The four isomers of *cis*-dihydrotetrabenazine are approximately equipotent as ligands for sigma-2 but isomers B and D bind more strongly to the sigma-1 receptor than do isomers A and C.

15 It is envisaged that, on the basis of their sigma receptor binding activities, the *cis*-dihydrotetrabenazine compounds of the invention will be useful in inhibiting or preventing the proliferation of tumour cells.

The ability of the compounds to inhibit or prevent the proliferation of tumour cells can be determined by testing the compounds against various tumour cell lines, for example as described in Example 6 below or using the methods described in Spruce *et al.*, *Cancer Research*, (2004), 64: 4875-4886.

In addition to *in vitro* studies on cell lines, the compounds of the invention may also be tested in *in vivo* in human tumour xenographs in immuno-compromised mice, for example as described in Spruce *et al.*, (2004), page 487.

25 It is also envisaged that the compounds of the invention will be active as anti-inflammatory agents. The anti-inflammatory activities of the compounds can be tested using a variety of methods well known to the skilled person.

The ability of the compounds of the invention to treat arthritis can be demonstrated in any one or more of the following test assays and models:

- (i) A murine collagen-induced arthritis model as described in Kakimoto *et al.*, *Cell Immunol.* 142: 326-337, 1992.
- (ii) A rat collagen-induced arthritis model as described in Knoerzer *et al.*, *Toxicol. Pathol.* 25:13-19, 1997.
- 5 (iii) A rat adjuvant arthritis model as described in Halloran *et al.*, *Arthritis Rheum.* 39: 810-819, 1996.
- (iv) A rat streptococcal cell wall-induced arthritis model as described in Schimmer, *et al.*, *J. Immunol.* 160: 1466-1477, 1998.
- (v) A SCID-mouse human rheumatoid arthritis model as described in Oppenheimer-
10 Marks *et al.*, *J. Clin. Invest.* 101: 1261-1272, 1998.
- The ability of compounds of the invention to treat inflammatory lung injury can be demonstrated by the following assays and test models:
- (vi) A murine oxygen-induced lung injury model as described in Wegner *et al.*, *Lung* 170:267-279, 1992.
- 15 (vii) A murine immune complex-induced lung injury model as described in Mulligan *et al.*, *J. Immunol.* 154:1350-1363, 1995.
- (viii) A murine acid-induced lung injury model as described in Nagase *et al.*, *Am. J. Respir. Crit. Care Med.* 154:504-510, 1996.

- The ability of the compounds of the invention to treat inflammatory bowel disease
20 can be demonstrated in a rabbit chemical-induced colitis model as described in Bennet *et al.*, *J. Pharmacol. Exp. Ther.* 280:988-1000, 1997.

The ability of compounds of the invention to treat inflammatory liver injury can be demonstrated in a murine liver injury model as described in Tanaka *et al.*, *J. Immunol.* 151:5088-5095, 1993.

The ability of compounds of the invention to treat inflammatory glomerular injury can be demonstrated in a rat nephrotoxic serum nephritis model as described in Kawasaki, *et al.*, *J. Immunol.* 150:1074-1083, 1993.

- 5 The anti-inflammatory activity of the compounds of the invention is also indicated by their ability to reduce the production of pro-inflammatory cytokines and inhibit T-cell proliferation as described in the Examples below.

Pharmaceutical Formulations

The dihydrotetrazine compounds are typically administered in the form of pharmaceutical compositions.

- 10 The pharmaceutical compositions can be in any form suitable for oral, parenteral, topical, intranasal, intrabronchial, ophthalmic, otic, rectal, intra-vaginal, or transdermal administration. Where the compositions are intended for parenteral administration, they can be formulated for intravenous, intramuscular, intraperitoneal, subcutaneous administration or for direct delivery into a target
15 organ or tissue by injection, infusion or other means of delivery.

Pharmaceutical dosage forms suitable for oral administration include tablets, capsules, caplets, pills, lozenges, syrups, solutions, sprays, powders, granules, elixirs and suspensions, sublingual tablets, sprays, wafers or patches and buccal patches.

- 20 Pharmaceutical compositions containing the dihydrotetrazine compounds of the invention can be formulated in accordance with known techniques, see for example, Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA, USA.

- 25 Thus, tablet compositions can contain a unit dosage of active compound together with an inert diluent or carrier such as a sugar or sugar alcohol, e.g.; lactose, sucrose, sorbitol or mannitol; and/or a non-sugar derived diluent such as sodium carbonate, calcium phosphate, talc, calcium carbonate, or a cellulose or derivative thereof such as methyl cellulose, ethyl cellulose, hydroxypropyl methyl cellulose, and starches such as corn starch. Tablets may also contain such standard

ingredients as binding and granulating agents such as polyvinylpyrrolidone, disintegrants (e.g. swellable crosslinked polymers such as crosslinked carboxymethylcellulose), lubricating agents (e.g. stearates), preservatives (e.g. parabens), antioxidants (e.g. BHT), buffering agents (for example phosphate or citrate buffers), and effervescent agents such as citrate/bicarbonate mixtures. Such excipients are well known and do not need to be discussed in detail here.

Capsule formulations may be of the hard gelatin or soft gelatin variety and can contain the active component in solid, semi-solid, or liquid form. Gelatin capsules can be formed from animal gelatin or synthetic or plant derived equivalents thereof.

10 The solid dosage forms (e.g.; tablets, capsules etc.) can be coated or un-coated, but typically have a coating, for example a protective film coating (e.g. a wax or varnish) or a release controlling coating. The coating (e.g. a Eudragit™ type polymer) can be designed to release the active component at a desired location within the gastro-intestinal tract. Thus, the coating can be selected so as to degrade
15 under certain pH conditions within the gastrointestinal tract, thereby selectively release the compound in the stomach or in the ileum or duodenum.

Instead of, or in addition to, a coating, the drug can be presented in a solid matrix comprising a release controlling agent, for example a release delaying agent which may be adapted to selectively release the compound under conditions of varying
20 acidity or alkalinity in the gastrointestinal tract. Alternatively, the matrix material or release retarding coating can take the form of an erodible polymer (e.g. a maleic anhydride polymer) which is substantially continuously eroded as the dosage form passes through the gastrointestinal tract.

Compositions for topical use include ointments, creams, sprays, patches, gels, liquid drops and inserts (for example intraocular inserts). Such compositions can be
25 formulated in accordance with known methods.

Compositions for parenteral administration are typically presented as sterile aqueous or oily solutions or fine suspensions, or may be provided in finely divided sterile powder form for making up extemporaneously with sterile water for
30 injection.

Examples of formulations for rectal or intra-vaginal administration include pessaries and suppositories which may be, for example, formed from a shaped mouldable or waxy material containing the active compound.

5 Compositions for administration by inhalation may take the form of inhalable powder compositions or liquid or powder sprays, and can be administered in standard form using powder inhaler devices or aerosol dispensing devices. Such devices are well known. For administration by inhalation, the powdered formulations typically comprise the active compound together with an inert solid powdered diluent such as lactose.

10 The compounds of the inventions will generally be presented in unit dosage form and, as such, will typically contain sufficient compound to provide a desired level of biological activity. For example, a formulation intended for oral administration may contain from 2 milligrams to 200 milligrams of active ingredient, more usually from 10 milligrams to 100 milligrams, for example, 12.5 milligrams, 25 milligrams
15 and 50 milligrams.

The active compound will be administered to a patient in need thereof (for example a human or animal patient) in an amount sufficient to achieve the desired therapeutic effect.

20 The subject in need of such administration is typically a patient suffering from or at risk of suffering from a proliferative disease such as cancer or an inflammatory disease as hereinbefore defined.

The compounds will typically be administered in amounts that are therapeutically or prophylactically useful and which generally are non-toxic. However, in certain situations, particularly in the case of cancer treatment, the benefits of administering
25 a dihydrotetrabenazine compound of the invention may outweigh the disadvantages of any toxic effects or side effects, in which case it may be considered desirable to administer compounds in amounts that are associated with a degree of toxicity.

A typical daily dose of the compound can be up to 1000 mg per day, for example in the range from 0.01 milligrams to 10 milligrams per kilogram of body weight, more

usually from 0.025 milligrams to 5 milligrams per kilogram of body weight, for example up to 3 milligrams per kilogram of bodyweight, and more typically 0.15 milligrams to 5 milligrams per kilogram of bodyweight although higher or lower doses may be administered where required.

5 For the treatment of cancers, the compounds of the formula (I) can be administered as the sole therapeutic agent or they can be administered in combination therapy with one of more other compounds for treatment of a particular disease state, for example a cancer as hereinbefore defined. Examples of other therapeutic agents and methods that may be used or administered together (whether concurrently or at
10 different time intervals) with the compounds of the formula (I) include but are not limited to:

- Topoisomerase I inhibitors (for example camptothecin compounds such as topotecan (Hycamtin), irinotecan and CPT11 (Camptosar).
- Antimetabolites (for example, anti-tumour nucleosides such as 5 --
15 fluorouracil, gemcitabine (Gemzar), raltitrexed (Tomudex), capecitabine (Xeloda), pemetrexed (Alimta), cytarabine or cytosine arabinoside or arabinosylcytosine [AraC] (Cytosar®), methotrexate (Matrex), fludarabine (Fludara) and tegafur.
- Tubulin targeting agents (for example, vinca alkaloids, vinblastine and
20 taxane compounds such as vincristine (Oncovin), vinorelbine (Navelbine), vinblastine (Velbe), paclitaxel (Taxol) and docetaxel (Taxotere).
- DNA binder and topo II inhibitors (for example, podophyllo – toxin derivatives and anthracycline derivatives such as etoposide (Eposin, Etophos, Vepesid, VP-16), teniposide (Vumon), daunorubicin (Cenubidine,
25 DaunoXome), epirubicin (Pharmorubicin), doxorubicin (Adriamycin; Doxil; Rubex), idarubicin (Zavedos), pegylated liposomal doxorubicin hydrochloride (Caelyx), liposome encapsulated doxorubicin citrate (Myocet), mitoxantrone (Novatrone, Onkotrone)
- Alkylating Agents (for example, nitrogen mustard or nitrosourea alkylating
30 agents and aziridines such as cyclophosphamide (Endoxana), melphalan (Alkeran), chlorambucil (Leukeran), busulphan (Myleran), carmustine

(BICNU), lomustine (CCNU), ifosfamide (Mitoxana), mitomycin (Mitomycin C Kyoma).

- Alkylating Agents (for example, platinum compounds such as cisplatin, carboplatin (Paraplatin) and oxaliplatin (Eloxatin)
- 5 • Monoclonal Antibodies (for example, the EGF family and its receptors and the VEGF family and its receptors, more particularly trastuzumab (Herceptin), cetuximab (Erbix), rituximab (Mabthera), tosihumomab (Bexxar), gemtuzumab ozogamicin (Mylotarg) and bevacizumab (Avastin).
- Anti-Hormones (for example anti-androgens including anti-estrogen agents
10 (e.g. aromatase inhibitors) such as tamoxifen (Nolvadex D, Soltamox, Tamofen), fulvestrant (Faslodex), raloxifene (Evista), toremifene (Fareston), droloxifene, letrozole (Femara), anastrozole (Arimidex), exemestane (Aromasin), vorozole (Rivizer), bicalutamide (Casodex, Cosudex), luprolide (Zoladex), megestrol acetate (Megace), aminoglutethimide (Cytadren) and
15 hexarotene (Targretin).
- Signal Transduction Inhibitors (such as gefitinib (Iressa), imatinib (Gleevec), erlotinib (Tarceva) and celecoxib (Celebrex).
- Proteasome Inhibitors such as bortezomib (Velcade)
- DNA methyl transferases such as temozolomide (Temodar)
- 20 • Cytokines and retinoids such as interferon alpha (IntronA, Roferon -A), interleukin 2 (Aldesleukin, Proleukin) and all trans-retinoic acid [ATRA] or tretinoin (Vesanoid).
- Radiotherapy.

Where the compounds of the invention are administered together with other
25 therapeutic agents or therapeutic methods in a combination therapy, the two or more treatments may be given in individually varying dose schedules and via different routes.

Where the compound of the invention is administered in combination therapy with one or more other therapeutic agents, the compounds can be administered

simultaneously or sequentially. When administered sequentially, they can be administered at closely spaced intervals (for example over a period of 5-10 minutes) or at longer intervals (for example 1, 2, 3, 4 or more hours apart, or even longer periods apart where required), the precise dosage regimen being commensurate with the properties of the therapeutic agent(s).

The compounds of the invention may also be administered in conjunction with non-chemotherapeutic treatments such as radiotherapy, photodynamic therapy, gene therapy; surgery and controlled diets.

For use in combination therapy with another chemotherapeutic agent, the compound of the invention and one, two, three, four or more other therapeutic agents can be, for example, formulated together in a dosage form containing two, three, four or more therapeutic agents. In an alternative, the individual therapeutic agents may be formulated separately and presented together in the form of a kit, optionally with instructions for their use.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the effect of the compounds of the invention, Isomer B (RU350) and Isomer D (RU346), on the production of TNF α by monocytes.

Figure 2 illustrates the effect of the compounds of the invention, Isomer B (RU350) and Isomer D (RU346), on the production of IL-4 by monocytes.

Figure 3 illustrates the effect of the compounds of the invention, Isomer B (RU350) and Isomer D (RU346), on the production of IL-2 by human monocytes.

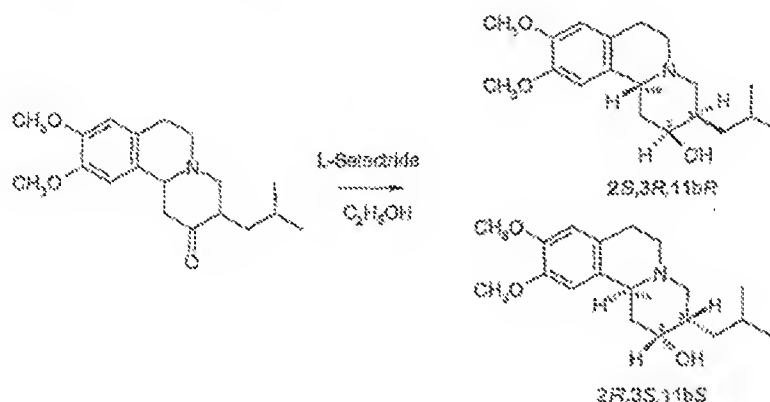
Figure 4 illustrates the effect of the compounds of the invention, Isomer B (RU350) and Isomer D (RU346), on the production of IL-5 by human monocytes.

Figure 5 illustrates the effect of the compounds of the invention, Isomer B (RU350) and Isomer D (RU346), on the production of IL-10 by human monocytes.

Figure 6 illustrates the effect of the compounds of the invention, Isomer B (RU350) and Isomer D (RU346), on the production of IL-12 by human monocytes.

EXAMPLES

The following non-limiting examples illustrate the synthesis and properties of the dihydrotetrabenazine compounds of the invention.

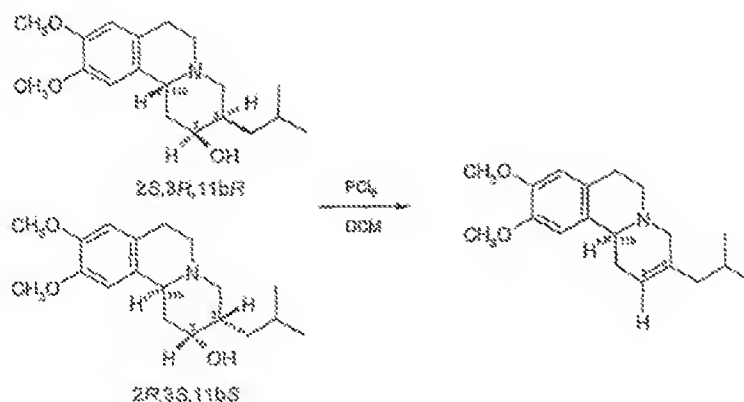
EXAMPLE 15 Preparation of 2*S*,3*S*,11*bR* and 2*R*,3*R*,11*bS* Isomers of Dihydrotetrabenazine1A. Reduction of *RR/SS* Tetrabenazine

1M L-Selectride[®] in tetrahydrofuran (135 ml, 135 mmol, 2.87 eq.) was added slowly over 30 minutes to a stirred solution of tetrabenazine *RR/SS* racemate (15 g, 47 mmol) in ethanol (75 ml) and tetrahydrofuran (75 ml) at 0 °C. After addition was complete the mixture was stirred at 0 °C for 30 minutes and then allowed to warm to room temperature.

The mixture was poured onto crushed ice (300 g) and water (100 ml) added. The solution was extracted with diethyl ether (2 x 200 ml) and the combined ethereal extracts washed with water (100 ml) and partly dried over anhydrous potassium carbonate. Drying was completed using anhydrous magnesium sulphate and, after filtration, the solvent was removed at reduced pressure (shielded from the light, bath temperature <20 °C) to afford a pale yellow solid.

The solid was slurried with petroleum ether (30-40 °C) and filtered to afford a white powdery solid (12 g, 80%).

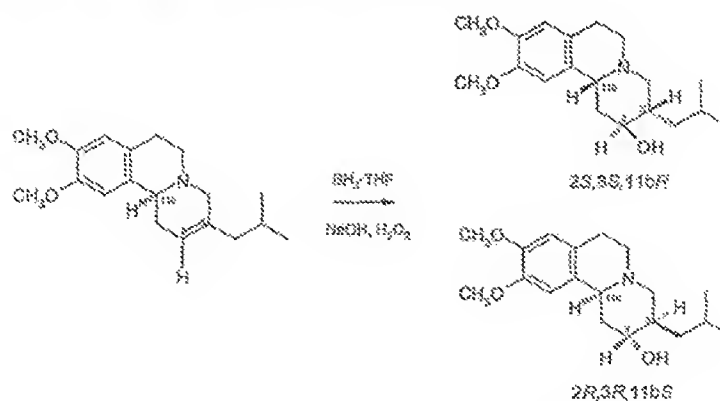
1B. Dehydration of reduced Tetrabenazine



Phosphorous pentachloride (32.8 g, 157.5 mmol, 2.5 eq) was added in portions over 30 minutes to a stirred solution of the reduced tetrahydroquinoline product from Example 1A (20 g, 62.7 mmol) in dichloromethane (200 ml) at 0 °C. After the addition was complete, the reaction mixture was stirred at 0 °C for a further 30 minutes and the solution poured slowly into 2M aqueous sodium carbonate solution containing crushed ice (0 °C). Once the initial acid gas evolution had ceased the mixture was basified (ca. pH 12) using solid sodium carbonate.

The alkaline solution was extracted using ethyl acetate (800 ml) and the combined organic extracts dried over anhydrous magnesium sulphate. After filtration the solvent was removed at reduced pressure to afford a brown oil, which was purified by column chromatography (silica, ethyl acetate) to afford the semi-pure alkene as a yellow solid (10.87 g, 58%).

1C. Hydration of the Crude Alkene from Example 1B

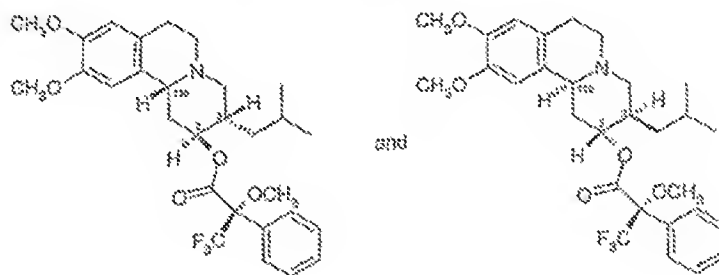


A solution of the crude alkene (10.87 g, 36.11 mmol) from Example 1B in dry THF (52 ml) at room temperature was treated with 1M borane-THF (155.6 ml, 155.6 mmol, 4.30 eq) added in a dropwise manner. The reaction was stirred for 2 hours, water (20 ml) was added and the solution basified to pH 12 with 30% aqueous sodium hydroxide solution.

Aqueous 30% hydrogen peroxide solution (30 ml) was added to the stirred alkaline reaction mixture and the solution was heated to reflux for 1 hour before being allowed to cool. Water (100 ml) was added and the mixture extracted with ethyl acetate (3 x 250 ml). The organic extracts were combined and dried over anhydrous magnesium sulphate and after filtration the solvent was removed at reduced pressure to afford a yellow oil (9 g).

The oil was purified using preparative HPLC (Column: Lichrospher Si60, 5 μ m, 250 x 21.20 mm, mobile phase: hexane : ethanol : dichloromethane (85:15:5); UV 254 nm, flow: 10 ml min⁻¹) at 350 mg per injection followed by concentration of the fractions of interest under vacuum. The product oil was then dissolved in ether and concentrated once more under vacuum to give the dihydrotetrabenazine racemate shown above as a yellow foam (5.76 g, 50%).

1D. Preparation of Mosher's ester derivatives



R-(+)- α -methoxy- α -trifluoromethylphenyl acetic acid (5 g, 21.35 mmol), oxalyl chloride (2.02 ml) and DMF (0.16 ml) were added to anhydrous dichloromethane (50 ml) and the solution was stirred at room temperature for 45 minutes. The solution was concentrated under reduced pressure and the residue was taken up in anhydrous dichloromethane (50 ml) once more. The resulting solution was cooled using an ice-water bath and dimethylaminopyridine (3.83 g, 31.34 mmol) was

added followed by a pre-dried solution (over 4Å sieves) in anhydrous dichloromethane of the solid product of Example 1C (5 g, 15.6 mmol). After stirring at room temperature for 45 minutes, water (234 ml) was added and the mixture extracted with ether (2 x 200 ml). The ether extract was dried over anhydrous magnesium sulphate, passed through a pad of silica and the product eluted using ether.

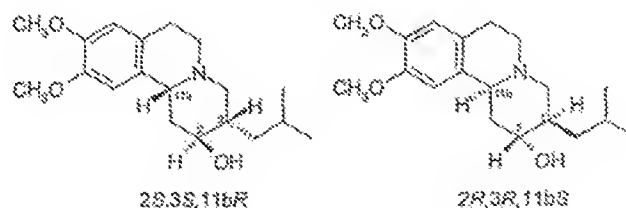
The collected ether eluate was concentrated under reduced pressure to afford an oil which was purified using column chromatography (silica, hexane : ether (10:1)). Evaporation of the collected column fractions of interest and removal of the solvent at reduced pressure gave a solid which was further purified using column chromatography (silica, hexane : ethyl acetate (1:1)) to give three main components which were partially resolved into Mosher's ester peaks 1 and 2.

Preparative HPLC of the three components (Column: 2 x Lichrospher Si60, 5 µm, 250 x 21.20 mm, mobile phase: hexane : isopropanol (97:3), UV 254 nm; flow: 10 ml min⁻¹) at 300 mg loading followed by concentration of the fractions of interest under vacuum gave the pure Mosher's ester derivatives

Peak 1 (3.89 g, 46.5%)

Peak 2 (2.78 g, 33%)

The fractions corresponding to the two peaks were subjected to hydrolysis to liberate the individual dihydrotetrabenazine isomers identified and characterised as Isomers A and B. Isomers A and B are each believed to have one of the following structures



More specifically, Isomer B is believed to have the 2*S*, 3*S*, 11*b**R* absolute configuration on the basis of the X-ray crystallography experiments described in Example 4 below.

1E. Hydrolysis of Peak 1 to give Isomer A

Aqueous 20% sodium hydroxide solution (87.5 ml) was added to a solution of Mosher's ester peak 1 (3.89 g, 7.27 mmol) in methanol (260 ml) and the mixture stirred and heated to reflux for 150 minutes. After cooling to room temperature
5 water (200 ml) was added and the solution extracted with ether (600 ml), dried over anhydrous magnesium sulphate and after filtration, concentrated under reduced pressure.

The residue was dissolved using ethyl acetate (200 ml), the solution washed with water (2 x 50 ml), the organic phase dried over anhydrous magnesium sulphate and
10 after filtration, concentrated under reduced pressure to give a yellow foam. This material was purified by column chromatography (silica, gradient elution of ethyl acetate : hexane (1:1) to ethyl acetate). The fractions of interest were combined and the solvent removed at reduced pressure. The residue was taken up in ether and the solvent removed at reduced pressure once more to give Isomer A as an off-white
15 foam (1.1 g, 47%).

Isomer A, which is believed to have the 2*R*,3*R*,11*bS* configuration (the absolute stereochemistry was not determined), was characterized by ¹H-NMR, ¹³C-NMR, IR, mass spectrometry, chiral HPLC and ORD. The IR, NMR and MS data for isomer A are set out in Table 1 and the Chiral HPLC and ORD data are set out in
20 Table 3.

1F. Hydrolysis of Peak 2 to give Isomer B

Aqueous 20% sodium hydroxide solution (62.5 ml) was added to a solution of Mosher's ester peak 2 (2.78 g, 5.19 mmol) in methanol (185 ml) and the mixture stirred and heated to reflux for 150 minutes. After cooling to room temperature
25 water (142 ml) was added and the solution extracted with ether (440 ml), dried over anhydrous magnesium sulphate and after filtration, concentrated under reduced pressure.

The residue was dissolved using ethyl acetate (200 ml), the solution washed with water (2 x 50 ml), the organic phase dried over anhydrous magnesium sulphate and

after filtration, concentrated under reduced pressure. Petroleum ether (30-40 °C) was added to the residue and the solution concentrated under vacuum once more to give Isomer B as a white foam (1.34 g, 81%).

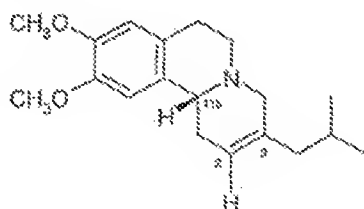
Isomer B, which is believed to have the 2*S*,3*S*,11*bR* configuration, was characterized by ¹H-NMR, ¹³C-NMR, IR, mass spectrometry, chiral HPLC, ORD and X-ray crystallography. The IR, NMR and MS data for Isomer B are set out in Table 1 and the Chiral HPLC and ORD data are set out in Table 3. The X-ray crystallography data are set out in Example 4.

EXAMPLE 2

10 Preparation of 2*R*,3*S*,11*bR* and 2*S*,3*R*,11*bS* Isomers of Dihydrotetrabenazine

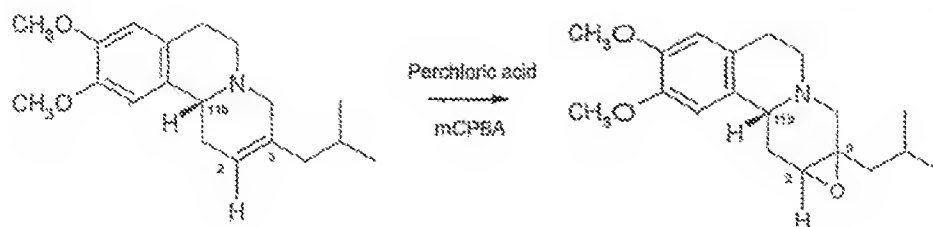
2A. Preparation of 2,3-Dehydrotetrabenazine

A solution containing a racemic mixture (15 g, 47 mmol) of *RR* and *SS* tetrabenazine enantiomers in tetrahydrofuran was subjected to reduction with L-Selectride® by the method of Example 1A to give a mixture of the 2*S*,3*R*,11*bR* and
15 2*R*,3*S*,11*bS* enantiomers of dihydrotetrabenazine as a white powdery solid (12 g, 80%). The partially purified dihydrotetrabenazine was then dehydrated using PCl₅ according to the method of Example 1B to give a semi-pure mixture of 11*bR* and 11*bS* isomers of 2,3-dehydrotetrabenazine (the 11*bR* enantiomer of which is shown below) as a yellow solid (12.92 g, 68%).



20

2B. Epoxidation of the Crude Alkene from Example 2A

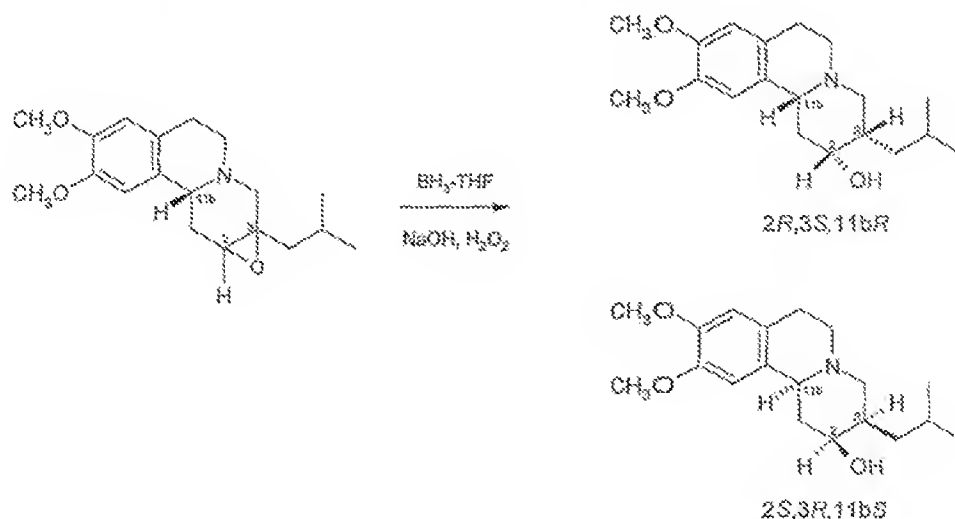


To a stirred solution of the crude alkene from Example 2A (12.92 g, 42.9 mmol) in methanol (215 ml) was added a solution of 70% perchloric acid (3.70 ml, 43 mmol) in methanol (215 ml). 77% 3-Chloroperoxybenzoic acid (15.50 g, 65 mmol) was added to the reaction and the resulting mixture was stirred for 18 hours at room temperature protected from light.

The reaction mixture was poured into saturated aqueous sodium sulphite solution (200 ml) and water (200 ml) added. Chloroform (300 ml) was added to the resulting emulsion and the mixture basified with saturated aqueous sodium bicarbonate (400 ml).

The organic layer was collected and the aqueous phase washed with additional chloroform (2 x 150 ml). The combined chloroform layers were dried over anhydrous magnesium sulphate and after filtration the solvent was removed at reduced pressure to give a brown oil (14.35 g, yield > 100% - probable solvent remains in product). This material was used without further purification.

2C. Reductive Ring Opening of the Epoxide from 2B



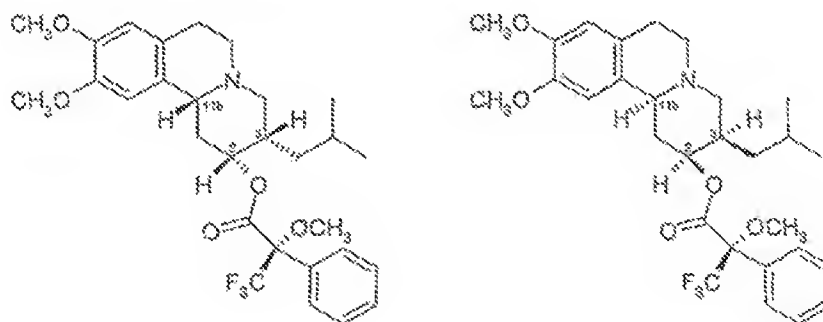
A stirred solution of the crude epoxide from Example 2B (14.35 g, 42.9 mmol, assuming 100% yield) in dry THF (80 ml) was treated slowly with 1M borane/THF (184.6 ml, 184.6 mmol) over 15 minutes. The reaction was stirred for two hours, water (65 ml) was added and the solution heated with stirring to reflux for 30 minutes.

After cooling, 30% sodium hydroxide solution (97 ml) was added to the reaction mixture followed by 30% hydrogen peroxide solution (48.6 ml) and the reaction was stirred and heated to reflux for an additional 1 hour.

The cooled reaction mixture was extracted with ethyl acetate (500 ml) dried over anhydrous magnesium sulphate and after filtration the solvent was removed at reduced pressure to give an oil. Hexane (230 ml) was added to the oil and the solution re-concentrated under reduced pressure.

The oily residue was purified by column chromatography (silica, ethyl acetate). The fractions of interest were combined and the solvent removed under reduced pressure. The residue was purified once more using column chromatography (silica, gradient, hexane to ether). The fractions of interest were combined and the solvents evaporated at reduced pressure to give a pale yellow solid (5.18 g, 38%).

2D. Preparation of Mosher's ester derivatives of the 2*R*,3*S*,11*bR* and 2*S*,3*R*,11*bS* Isomers of Dihydrotetrahenazine



R-(+)- α -methoxy- α -trifluoromethylphenyl acetic acid (4.68 g, 19.98 mmol), oxalyl chloride (1.90 ml) and DMF (0.13 ml) were added to anhydrous dichloromethane (46 ml) and the solution stirred at room temperature for 45 minutes. The solution was concentrated under reduced pressure and the residue was taken up in anhydrous

dichloromethane (40 ml) once more. The resulting solution was cooled using an ice-water bath and dimethylaminopyridine (3.65 g, 29.87 mmol) was added followed by a pre-dried solution (over 4Å sieves) in anhydrous dichloromethane (20 ml) of the solid product of Example 2C (4.68 g, 14.6 mmol). After stirring at
 5 room temperature for 45 minutes, water (234 ml) was added and the mixture extracted with ether (2 x 200 ml). The ether extract was dried over anhydrous magnesium sulphate, passed through a pad of silica and the product eluted using ether.

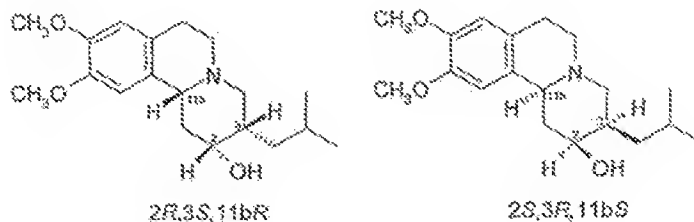
The collected ether eluate was concentrated under reduced pressure to afford an oil
 10 which was purified using column chromatography (silica, hexane : ether (1:1)). Evaporation of the collected column fractions of interest and removal of the solvent at reduced pressure gave a pink solid (6.53 g)

Preparative HPLC of the solid (Column: 2 x Lichrospher Si60, 5 µm, 250 x 21.20 mm; mobile phase hexane : isopropanol (97:3); UV 254 nm; flow: 10 ml min⁻¹) at
 15 100 mg loading followed by concentration of the fractions of interest under vacuum gave a solid which was slurried with petroleum ether (30-40 °C) and collected by filtration to give the pure Mosher's ester derivatives

Peak 1 (2.37 g, 30%)

Peak 2 (2.42 g, 30%)

20 The fractions corresponding to the two peaks were subjected to hydrolysis to liberate the individual dihydrotetrabenazine isomers identified and characterised as Isomers C and D. Isomers C and D are each believed to have one of the following structures



25 2F. Hydrolysis of Peak 1 to give Isomer C

- 20% aqueous sodium hydroxide solution (53 ml) was added to a stirred solution of Mosher's ester peak 1 (2.37 g, 4.43 mmol) in methanol (158 ml) and the mixture stirred at reflux for 150 minutes. After cooling water (88 ml) was added to the reaction mixture and the resulting solution extracted with ether (576 ml). The organic extract was dried over anhydrous magnesium sulphate and after filtration the solvent removed at reduced pressure. Ethyl acetate (200 ml) was added to the residue and the solution washed with water (2 x 50 ml). The organic solution was dried over anhydrous magnesium sulphate and after filtration the solvent removed at reduced pressure.
- 10 This residue was treated with petroleum ether (30-40 °C) and the resulting suspended solid collected by filtration. The filtrate was concentrated at reduced pressure and the second batch of suspended solid was collected by filtration. Both collected solids were combined and dried under reduced pressure to give Isomer C (1.0 g, 70%).
- 15 Isomer C, which is believed to have either the 2*R*,3*S*,11*bR* or 2*S*,3*R*,11*bS* configuration (the absolute stereochemistry was not determined), was characterized by ¹H-NMR, ¹³C-NMR, IR, mass spectrometry, chiral HPLC and ORD. The IR, NMR and MS data for Isomer C are set out in Table 2 and the Chiral HPLC and ORD data are set out in Table 4.
- 20 2G. Hydrolysis of Peak 2 to give Isomer D
- 20% aqueous sodium hydroxide solution (53 ml) was added to a stirred solution of Mosher's ester peak 2 (2.42 g, 4.52 mmol) in methanol (158 ml) and the mixture stirred at reflux for 150 minutes. After cooling water (88 ml) was added to the reaction mixture and the resulting solution extracted with ether (576 ml). The organic extract was dried over anhydrous magnesium sulphate and after filtration the solvent removed at reduced pressure. Ethyl acetate (200 ml) was added to the residue and the solution washed with water (2 x 50 ml). The organic solution was dried over anhydrous magnesium sulphate and after filtration the solvent removed at reduced pressure.

This residue was treated with petroleum ether (30-40 °C) and the resulting suspended orange solid collected by filtration. The solid was dissolved in ethyl acetate : hexane (15:85) and purified by column chromatography (silica, gradient ethyl acetate : hexane (15:85) to ethyl acetate). The fractions of interest were
 5 combined and the solvent removed at reduced pressure. The residue was slurried with petroleum ether (30-40 °C) and the resulting suspension collected by filtration. The collected solid was dried under reduced pressure to give Isomer D as a white solid (0.93 g, 64%).

Isomer D, which is believed to have either the 2*R*,3*S*,11*bR* or 2*S*,3*R*,11*bS*
 10 configuration (the absolute stereochemistry was not determined), was characterized by ¹H-NMR, ¹³C-NMR, IR, mass spectrometry, chiral HPLC and ORD. The IR, NMR and MS data for Isomer D are set out in Table 2 and the Chiral HPLC and ORD data are set out in Table 4.

In Tables 1 and 2, the infra red spectra were determined using the KBr disc method.
 15 The ¹H NMR spectra were carried out on solutions in deuterated chloroform using a Varian Gemini NMR spectrometer (200 MHz.). The ¹³C NMR spectra were carried out on solutions in deuterated chloroform using a Varian Gemini NMR spectrometer (50MHz). The mass spectra were obtained using a Micromass Platform II (ES⁺ conditions) spectrometer. In Tables 3 and 4, the Optical Rotatory
 20 Dispersion figures were obtained using an Optical Activity PolAAr 2001 instrument in methanol solution at 24°C. The HPLC retention time measurements were carried out using an HP1050 HPLC chromatograph with UV detection.

Tables 1 and 2 – Spectroscopic Data

Table 1				
Dihydrotetrabenazine isomer	¹ H-NMR spectrum (CDCl ₃)	¹³ C-NMR spectrum (CDCl ₃)	IR Spectrum (KBr solid)	Mass Spectrum (ES ⁺)
Isomers A and B	6.67δ 1H (s); 6.57 δ 1H (s); 3.84 δ 6H (s);	147.7δ; 147.6δ; 130.5δ;	2950 cm ⁻¹ ; 2928 cm ⁻¹ ; 2868 cm ⁻¹ ;	MH ⁺ 320

Table 1

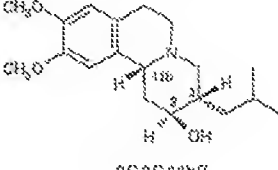
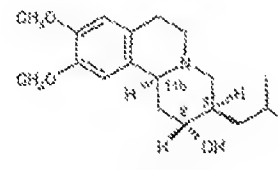
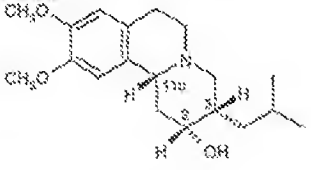
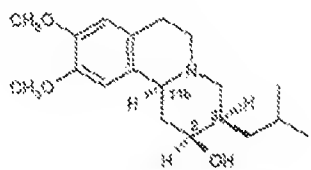
Dihydrotetrabenazine isomer	¹ H-NMR spectrum (CDCl ₃)	¹³ C-NMR spectrum (CDCl ₃)	IR Spectrum (KBr solid)	Mass Spectrum (ES ⁺)
 <p>2S,8S,11bR</p> <p>OR</p>  <p>2R,8R,11bS</p>	3.55 δ 1H (br. d); 3.08 δ 1H (m); 2.79 δ 2H (m); 2.55 δ 3H (m); 2.17 δ 1H (m); 1.72 δ 6H (m); 1.02 δ 1H (m); 0.88 δ 6H (s)	127.6δ; 112.1δ; 108.4δ; 70.5δ; 57.5 δ; 56.5 δ; 56.3 δ; 54.8 δ; 53.2 δ; 40.4 δ; 40.1 δ; 36.0 δ; 28.8 δ; 26.2 δ; 23.7δ; 22.9 δ	2834 cm ⁻¹ ; 1610 cm ⁻¹ ; 1511 cm ⁻¹ ; 1464 cm ⁻¹ ; 1364 cm ⁻¹ ; 1324 cm ⁻¹ ; 1258 cm ⁻¹ ; 1223 cm ⁻¹ ; 1208 cm ⁻¹ ; 1144 cm ⁻¹ ; 1045 cm ⁻¹ ; 1006 cm ⁻¹ ; 870 cm ⁻¹ ; 785 cm ⁻¹ ; 764 cm ⁻¹	

Table 2

Dihydrotetrabenazine isomer	¹ H-NMR spectrum (CDCl ₃)	¹³ C-NMR spectrum (CDCl ₃)	IR Spectrum (KBr solid)	Mass Spectrum (ES ⁺)
Isomers C and D	6.68 δ 1H (s); 6.58 δ 1H (s); 3.92 δ 1H (m); 3.84 δ 6H (s); 3.15 δ 1H (m); 2.87 δ 3H (m); 2.43 δ 4H (m); 1.81 δ 1H (m); 1.64 δ 4H (m); 1.21 δ 1H (m); 0.94 δ 3H (d);	147.8 δ; 147.7 δ; 130.4 δ; 127.2 δ; 112.0 δ; 108.3 δ; 72.4 δ; 61.2 δ; 58.3 δ; 56.5 δ; 56.3 δ;	3370 cm ⁻¹ ; 2950 cm ⁻¹ ; 2929 cm ⁻¹ ; 1611 cm ⁻¹ ; 1512 cm ⁻¹ ; 1463 cm ⁻¹ ; 1362 cm ⁻¹ ; 1334 cm ⁻¹ ; 1259 cm ⁻¹ ; 1227 cm ⁻¹ ; 1148 cm ⁻¹ ;	MH ⁺ 320

Table 2

Dihydrotetrabenazine isomer	¹ H-NMR spectrum (CDCl ₃)	¹³ C-NMR spectrum (CDCl ₃)	IR Spectrum (KBr solid)	Mass Spectrum (ES ⁺)
 <p>2R,3S,11bR</p> <p>OR</p>  <p>2S,3R,11bS</p>	0.89 δ 3H (d)	52.7 δ; 38.6 δ; 36.7 δ; 34.4 δ; 29.6 δ; 26.5 δ; 24.4 δ; 22.5 δ	1063 cm ⁻¹ ; 1024 cm ⁻¹ ; 855 cm ⁻¹ ; 766 cm ⁻¹	

Tables 3 and 4 -- Chromatography and ORD Data

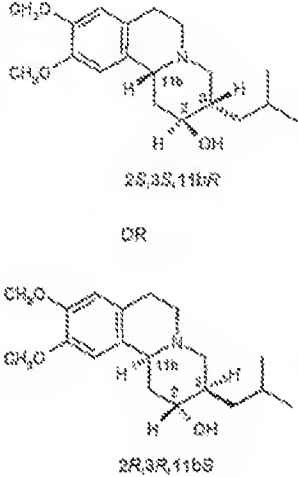
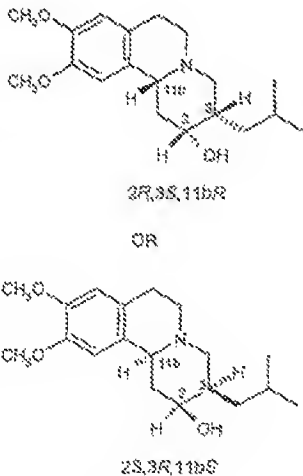
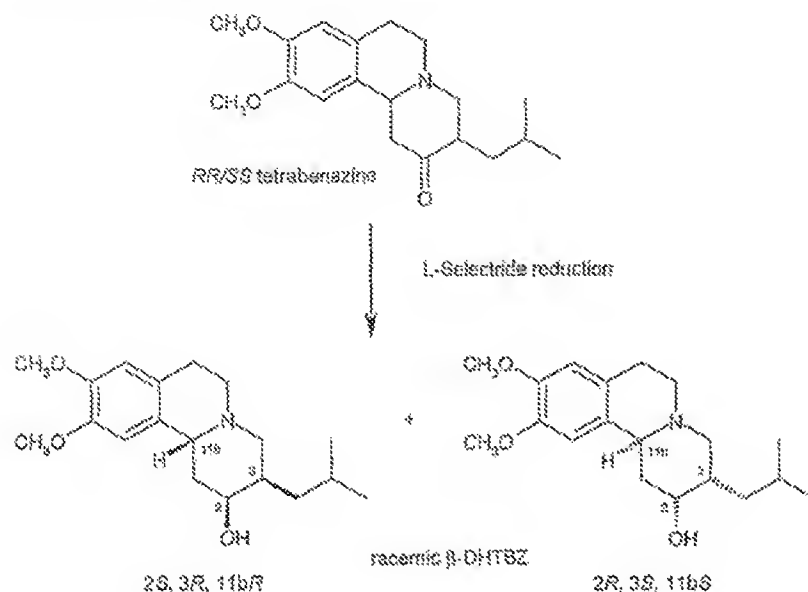
Table 3		
Dihydrotrabenazine isomer	Chiral HPLC Methods and Retention Times	ORD (MeOH, 21°C)
<p>Isomers A and B</p>  <p>2S,3S,11bR</p> <p>OR</p> <p>2R,3R,11bS</p>	<p>Column:</p> <p>Chirex (S)-VAL₂ (R)-NEA, 250 x 4.6 mm</p> <p>Mobile phase: Hexane : 1,2-dichloroethane : ethanol (36:62:2)</p> <p>Flow: 1.0 ml min⁻¹</p> <p>UV: 254 nm</p> <p>Retention times:</p> <p>Isomer A 16.6 min</p> <p>Isomer B 15.3 min</p>	<p>Isomer A</p> <p>[α_D] -114.6°</p> <p>Isomer B</p> <p>[α_D] +123°</p>

Table 4		
Isomers C and D	Column:	Isomer C
 <p>2R,3S,11bR</p> <p>OR</p> <p>2S,3R,11bS</p>	Chirex (S)-VAL ₂ (R)-NEA, 250 x 4.6mm	[α _D] +150.9°
	Mobile phase: Hexane : ethanol (92:8)	
	Flow: 1.0 ml min ⁻¹	Isomer D
	UV: 254 nm	[α _D] -145.7°
	Retention times:	
	Isomer C 20.3 min	
	Isomer D 19.4 min	

EXAMPLE 3Alternative Method of Preparation of Isomer B and Preparation of Mesylate Salt3A. Reduction of RR/SS Tetrabenazine

- 5 1M L-Selectride[®] in tetrahydrofuran (52 ml, 52 mmol, 1.1 eq) was added slowly over 30 minutes to a cooled (ice bath), stirred solution of tetrabenazine racemate (15 g, 47 mmol) in tetrahydrofuran (56 ml). After the addition was complete, the mixture was allowed to warm to room temperature and stirred for a further six hours. TLC analysis (silica, ethyl acetate) showed only very minor amounts of
- 10 starting material remained.

The mixture was poured on to a stirred mixture of crushed ice (112 g), water (56 ml) and glacial acetic acid (12.2 g). The resulting yellow solution was washed with ether (2 x 50 ml) and basified by the slow addition of solid sodium carbonate (ca. 13 g). Pet-ether (30-40 °C) (56 ml) was added to the mixture with stirring and the

15 crude β -DHTBZ was collected as a white solid by filtration.

The crude solid was dissolved in dichloromethane (ca. 150 ml) and the resulting solution washed with water (40 ml), dried using anhydrous magnesium sulphate, filtered and concentrated at reduced pressure to ca. 40 ml. A thick suspension of white solid was formed. Pet-ether (30-40 °C) (56 ml) was added and the

suspension was stirred for fifteen minutes at laboratory temperature. The product was collected by filtration and washed on the filter until snow-white using pet-ether (30-40°C) (40 to 60 ml) before air-drying at room temperature to yield β -DHTBZ (10.1 g, 67%) as a white solid. TLC analysis (silica, ethyl acetate) showed only one component.

3B. Preparation and Fractional Crystallisation of the Camphorsulphonic acid Salt of Racemic β -DHTBZ

The product of Example 3A and 1 equivalent of (S)-(+)-Camphor-10-sulphonic acid were dissolved with heating in the minimum amount of methanol. The resulting solution was allowed to cool and then diluted slowly with ether until formation of the resulting solid precipitation was complete. The resulting white crystalline solid was collected by filtration and washed with ether before drying.

The camphorsulphonic acid salt of (10 g) was dissolved in a mixture of hot absolute ethanol (170 ml) and methanol (30 ml). The resulting solution was stirred and allowed to cool. After two hours the precipitate formed was collected by filtration as a white crystalline solid (2.9 g). A sample of the crystalline material was shaken in a separating funnel with excess saturated aqueous sodium carbonate and dichloromethane. The organic phase was separated, dried over anhydrous magnesium sulphate, filtered and concentrated at reduced pressure. The residue was triturated using pet-ether (30-40 °C) and the organic solution concentrated once more. Chiral HPLC analysis of the salt using a Chirex (S)-VAL and (R)-NEA 250 x 4.6 mm column, and a hexane : ethanol (98:2) eluent at a flow rate of 1 ml/minute showed showed that the isolated β -DHTBZ was enriched in one enantiomer (e.e. ca. 80%).

The enriched camphorsulphonic acid salt (14 g) was dissolved in hot absolute ethanol (140 ml) and propan-2-ol (420 ml) was added. The resulting solution was stirred and a precipitate began to form within one minute. The mixture was allowed to cool to room temperature and stirred for one hour. The precipitate formed was collected by filtration, washed with ether and dried to give a white crystalline solid (12 g).

The crystalline material was shaken in a separating funnel with excess saturated aqueous sodium carbonate and dichloromethane. The organic phase was separated, dried over anhydrous magnesium sulphate, filtered and concentrated at reduced
5 pressure. The residue was triturated using pet-ether (30-40 °C) and the organic solution concentrated once more to yield (after drying in vacuo.) (+)- β -DHTBZ (6.6 g, ORD +107.8°). The isolated enantiomer has e.e. >97%.

3C. Preparation of Isomer B

A solution of phosphorus pentachloride (4.5 g, 21.6 mmol, 1.05 eq) in
10 dichloromethane (55 ml) was added steadily over ten minutes to a stirred, cooled (ice-water bath) solution of the product of Example 3B (6.6 g, 20.6 mmol) in dichloromethane (90 ml). When the addition was complete, the resulting yellow solution was stirred for a further ten minutes before pouring on to a rapidly stirred mixture of sodium carbonate (15 g) in water (90 ml) and crushed ice (90 g). The
15 mixture was stirred for a further 10 minutes and transferred to a separating funnel.

Once the phases had separated, the brown dichloromethane layer was removed, dried over anhydrous magnesium sulphate, filtered and concentrated at reduced pressure to give the crude alkene intermediate as brown oil (ca. 6.7 g). TLC analysis (silica, ethyl acetate) showed that no (+)- β -DHTBZ remained in the crude
20 product.

The crude alkene was taken up (dry nitrogen atmosphere) in anhydrous tetrahydrofuran (40 ml) and a solution of borane in THF (1 M solution, 2.5 eq, 52 ml) was added with stirring over fifteen minutes. The reaction mixture was then stirred at room temperature for two hours. TLC analysis (silica, ethyl acetate)
25 showed that no alkene intermediate remained in the reaction mixture.

A solution of sodium hydroxide (3.7 g) in water (10 ml) was added to the stirring reaction mixture, followed by an aqueous solution of hydrogen peroxide (50%, ca. 7 ml) and the two-phase mixture formed was stirred at reflux for one hour. TLC analysis of the organic phase at this time (silica, ethyl acetate) showed the

appearance of a product with R_f as expected for Isomer B. A characteristic non-polar component was also seen.

The reaction mixture was allowed to cool to room temperature and was poured into a separating funnel. The upper organic layer was removed and concentrated under reduced pressure to remove the majority of THF. The residue was taken up in ether (stabilised (BHT), 75 ml), washed with water (40 ml), dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to give a pale yellow oil (8.1 g).

The yellow oil was purified using column chromatography (silica, ethyl acetate : hexane (80:20), increasing to 100% ethyl acetate) and the desired column fractions collected, combined and concentrated at reduced pressure to give a pale oil which was treated with ether (stabilised, 18 ml) and concentrated at reduced pressure to give Isomer B as a pale yellow solid foam (2.2 g).

Chiral HPLC using the conditions set out in Example 3B confirmed that Isomer B had been produced in an enantiomeric excess (e.e.) of greater than 97%.

The optical rotation was measured using a Bellingham Stanley ADP220 polarimeter and gave an $[\alpha_D]$ of +123.5°.

3D. Preparation of the Mesylate salt of Isomer B

The methanesulphonate salt of Isomer B was prepared by dissolving a mixture of 1 equivalent of Isomer B from Example 3C and 1 equivalent of methane sulphonic acid in the minimum amount of ethanol and then adding diethyl ether. The resulting white precipitate that formed was collected by filtration and dried *in vacuo* to give the mesylate salt in a yield of ca. 85% and a purity (by HPLC) of ca. 96%.

EXAMPLE 4

X-Ray Crystallographic Studies on Isomer B

The (S)-(+)-Camphor-10-sulphonic acid salt of Isomer B was prepared and a single crystal was subjected to X-ray crystallographic studies under the following conditions:

Diffraction: Nonius KappaCCD area detector (1/1 scans and 0/1 scans to fill asymmetric unit).

Cell determination: DirAx (Duisenberg, A.J.M. (1992). *J. Appl. Cryst.* 25, 92-96.)

Data collection: Collect (Collect: Data collection software, R. Hooft, Nonius B. V.,
5 1998)

Data reduction and cell refinement: Demo (Z. Otwinowski & W. Minor, *Methods in Enzymology* (1997) Vol. 276: *Macromolecular Crystallography*, part A, pp. 307-326; C. W. Carter, Jr & R. M. Sweet, Eds., Academic Press).

Absorption correction: Sheldrick, G. M. SADABS - Bruker Nonius area detector
10 scaling and absorption correction - V2.10

Structure solution: SHELXS97 (G. M. Sheldrick, *Acta Cryst.* (1990) A46 467-473).

Structure refinement: SHELXL97 (G. M. Sheldrick (1997), University of Göttingen, Germany)

Graphics: Cameron - A Molecular Graphics Package (D. M. Watkin, L. Pearce and
15 C. K. Prout, Chemical Crystallography Laboratory, University of Oxford, 1993)

Special details: All hydrogen atoms were placed in idealised positions and refined using a riding model, except those of the NH and OH which were located in the difference map and refined using restraints. Chirality: N1=R, C12=S, C13=S, C15=R, C21=S, C24=R.

20 The results of the studies are set out below in Tables A, B, C, D and E.

In the Tables, the label RUS0350 refers to Isomer B.

TABLE A

Identification code	2005bdy0585 (RUS0350)
Empirical formula	$C_{29}H_{45}NO_7S$
Formula weight	551.72
Temperature	120(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	$a = 7.1732(9)$ Å $b = 12.941(2)$ Å $c = 31.025(4)$ Å
Volume	$2880.1(7)$ Å ³
Z	4
Density (calculated)	1.272 Mg / m ³
Absorption coefficient	0.158 mm ⁻¹
$F(000)$	1192
Crystal	Colourless Slab
Crystal size	$0.2 \times 0.2 \times 0.04$ mm ³
θ range for data collection	$3.06 - 27.37^\circ$
Index ranges	$-8 \leq h \leq 9, -16 \leq k \leq 16, -36 \leq l \leq 39$
Reflections collected	36802
Independent reflections	6326 [$R_{\sigma} = 0.0863$]
Completeness to $\theta = 27.37^\circ$	97.1 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9937 and 0.9690
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6326 / 1 / 357
Goodness-of-fit on F^2	1.042
Final R indices [$F^2 > 2\sigma(F^2)$]	$R1 = 0.0498, wR2 = 0.0967$
R indices (all data)	$R1 = 0.0901, wR2 = 0.1108$
Absolute structure parameter	0.04(8)
Extinction coefficient	0.0059(7)
Largest diff. peak and hole	0.236 and -0.336 e Å ⁻³

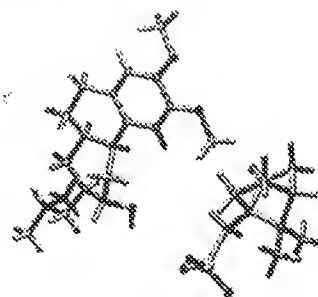


TABLE B. Atomic coordinates [$\times 10^4$], equivalent isotropic displacement parameters [$\text{\AA}^2 \times 10^3$] and site occupancy factors. U_{eq} is defined as one third of the trace of the orthogonalized U^{β} tensor.

	Atom	x	y	z	U_{eq}	S.o.f.
10	N1	4839(3)	11119(2)	2180(1)	24(1)	1
	O1	2515(3)	13171(1)	349(1)	31(1)	1
	O2	5581(3)	14030(1)	598(1)	32(1)	1
	O3	9220(3)	12834(2)	2385(1)	36(1)	1
	Cl	870(4)	12674(2)	190(1)	36(1)	1
15	C2	3176(3)	12838(2)	739(1)	25(1)	1

	C3	2346(4)	12109(2)	997(1)	25(1)	1
	C4	3124(3)	11821(2)	1395(1)	24(1)	1
	C5	4773(3)	12276(2)	1527(1)	23(1)	1
	C6	5629(4)	13024(2)	1262(1)	24(1)	1
5	C7	4861(4)	13308(2)	875(1)	25(1)	1
	C8	7189(4)	14582(2)	747(1)	38(1)	1
	C9	2182(3)	11023(2)	1673(1)	28(1)	1
	C10	2759(3)	11118(2)	2137(1)	26(1)	1
	C11	5366(3)	11096(2)	2656(1)	25(1)	1
10	C12	7292(4)	11536(2)	2747(1)	25(1)	1
	C13	7468(4)	12663(2)	2590(1)	25(1)	1
	C14	5988(4)	12911(2)	2252(1)	25(1)	1
	C15	5773(4)	12010(2)	1943(1)	24(1)	1
	C16	7734(4)	11477(2)	3232(1)	28(1)	1
15	C17	7752(4)	10418(2)	3449(1)	34(1)	1
	C18	9198(6)	9696(3)	3249(1)	65(1)	1
	C19	8114(4)	10562(2)	3930(1)	41(1)	1
	C20	7509(4)	8131(2)	1250(1)	31(1)	1
	S1	7409(1)	8792(1)	1754(1)	27(1)	1
20	O4	7758(2)	7965(1)	2064(1)	30(1)	1
	O5	8831(3)	9582(2)	1760(1)	49(1)	1
	O6	5524(2)	9221(1)	1798(1)	32(1)	1
	O7	7406(3)	6932(1)	498(1)	48(1)	1
	C21	6858(3)	8622(2)	830(1)	25(1)	1
25	C22	7154(4)	7851(2)	459(1)	30(1)	1
	C23	7073(4)	8450(2)	40(1)	32(1)	1
	C24	6848(3)	9544(2)	203(1)	28(1)	1
	C25	4742(3)	8877(2)	787(1)	29(1)	1
	C26	4742(3)	8877(2)	787(1)	29(1)	1
30	C27	7773(4)	9610(2)	630(1)	25(1)	1
	C28	7431(4)	10628(2)	868(1)	29(1)	1
	C29	9895(4)	9489(2)	569(1)	36(1)	1

TABLE C. Bond lengths [Å] and angles [°].

35	Ni-C10	1.498(3)	C14-C15	1.518(3)
	Ni-C15	1.522(3)	C16-C17	1.526(3)
	Ni-C11	1.524(3)	C17-C18	1.527(4)
	O1-C2	1.368(3)	C17-C19	1.527(4)
	O1-C1	1.432(3)	C20-C21	1.525(3)
40	O2-C7	1.369(3)	C20-S1	1.784(2)
	O2-C8	1.433(3)	Si-O5	1.4442(19)
	O3-C13	1.425(3)	Si-O4	1.4607(17)
	C2-C3	1.372(3)	Si-O6	1.4676(18)
	C2-C7	1.417(3)	O7-C22	1.208(3)
45	C3-C4	1.407(3)	C21-C22	1.537(4)
	C4-C5	1.384(3)	C21-C26	1.559(3)
	C4-C9	1.506(3)	C21-C27	1.565(3)

	C5-C6	1.411(3)	C22-C23	1.517(4)
	C5-C15	1.516(3)	C23-C24	1.535(4)
	C6-C7	1.372(3)	C24-C25	1.548(4)
	C9-C10	1.504(3)	C24-C27	1.554(4)
5	C11-C12	1.521(3)	C25-C26	1.557(4)
	C12-C16	1.540(3)	C27-C28	1.529(3)
	C12-C13	1.544(3)	C27-C29	1.542(4)
	C13-C14	1.524(3)		
10	C10-NI-C15	113.33(19)	C12-C11-NI	113.43(19)
	C10-NI-C11	109.46(18)	C11-C12-C16	110.5(2)
	C15-NI-C11	111.96(19)	C11-C12-C13	111.7(2)
	C2-O1-C1	116.6(2)	C16-C12-C13	109.84(19)
	C7-O2-C8	116.27(19)	O3-C13-C14	106.0(2)
15	O1-C2-C3	125.5(2)	O3-C13-C12	111.1(2)
	O1-C2-C7	115.0(2)	C14-C13-C12	111.0(2)
	C3-C2-C7	119.5(2)	C15-C14-C13	110.1(2)
	C2-C3-C4	121.5(2)	C5-C15-C14	114.3(2)
	C5-C4-C3	119.2(2)	C5-C15-NI	112.0(2)
20	C5-C4-C9	120.3(2)	C14-C15-NI	108.7(2)
	C3-C4-C9	120.5(2)	C17-C16-C12	118.4(2)
	C4-C5-C6	119.4(2)	C16-C17-C18	112.2(2)
	C4-C5-C15	124.1(2)	C16-C17-C19	108.7(2)
	C6-C5-C15	116.6(2)	C18-C17-C19	110.8(3)
25	C7-C6-C5	121.3(2)	C21-C20-S1	122.51(18)
	O2-C7-C6	125.4(2)	O5-S1-O4	112.93(11)
	O2-C7-C2	115.4(2)	O5-S1-O6	112.47(12)
	C6-C7-C2	119.2(2)	O4-S1-O6	111.93(11)
	C10-C9-C4	111.7(2)	O5-S1-C20	108.81(13)
30	NI-C10-C9	111.0(2)	O4-S1-C20	102.60(11)
	O6-S1-C20	107.44(12)	C23-C24-C25	106.4(2)
35	C20-C21-C22	109.0(2)	C23-C24-C27	103.3(2)
	C20-C21-C26	117.3(2)	C25-C24-C27	102.3(2)
	C22-C21-C26	102.1(2)	C24-C25-C26	102.9(2)
	C20-C21-C27	123.4(2)	C25-C26-C21	104.2(2)
	C22-C21-C27	100.21(19)	C28-C27-C29	107.8(2)
	C26-C21-C27	101.7(2)	C28-C27-C24	112.0(2)
40	O7-C22-C23	126.4(2)	C29-C27-C24	113.7(2)
	O7-C22-C21	125.9(2)	C28-C27-C21	116.5(2)
	C23-C22-C21	107.7(2)	C29-C27-C21	112.3(2)
	C22-C23-C24	101.3(2)	C24-C27-C21	94.27(19)

TABLE D. Anisotropic displacement parameters [$\text{\AA}^2 \times 10^3$]. The anisotropic displacement factor exponent takes the form: $-2\pi^2 [h^2 a^{*2} U^{11} + \dots + 2 h k a^* b^* U^{12}]$.

Atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
------	----------	----------	----------	----------	----------	----------

	Ni	26(1)	24(1)	23(1)	2(1)	-1(1)	-3(1)
	O1	37(1)	30(1)	24(1)	3(1)	-7(1)	-4(1)
	O2	41(1)	31(1)	25(1)	5(1)	-2(1)	-10(1)
	O3	26(1)	49(1)	32(1)	7(1)	-3(1)	-9(1)
5	Cl	41(2)	36(2)	32(2)	3(1)	-9(1)	-8(2)
	C2	30(2)	24(2)	22(1)	1(1)	-1(1)	2(1)
	C3	25(1)	26(1)	24(1)	-3(1)	-2(1)	2(1)
	C4	26(2)	22(1)	23(1)	-1(1)	2(1)	-1(1)
	C5	24(1)	22(1)	23(1)	-2(1)	1(1)	0(1)
10	C6	26(1)	22(1)	24(1)	-3(1)	2(1)	-5(1)
	C7	30(2)	22(1)	22(1)	2(1)	4(1)	-4(1)
	C8	45(2)	34(2)	36(2)	5(1)	-2(1)	-20(2)
	C9	23(1)	32(1)	29(2)	3(1)	-1(1)	-4(1)
	ClO	26(1)	29(1)	25(1)	2(1)	0(1)	-5(1)
15	C11	31(1)	25(1)	20(1)	2(1)	0(1)	-2(1)
	C12	26(1)	26(1)	23(1)	-1(1)	1(1)	-1(1)
	C13	26(1)	28(1)	23(1)	-1(1)	-1(1)	-2(1)
	C14	30(2)	22(2)	24(1)	-1(1)	1(1)	-1(1)
	C15	22(1)	22(1)	28(1)	2(1)	0(1)	-4(1)
20	C16	31(1)	28(1)	24(1)	-1(1)	-3(1)	3(1)
	C17	46(2)	31(2)	25(1)	1(1)	-7(1)	0(2)
	C18	106(3)	46(2)	41(2)	6(2)	-1(2)	31(2)
	C19	51(2)	41(2)	31(2)	9(2)	-7(1)	-4(2)
	C20	30(2)	34(2)	29(1)	2(1)	3(1)	9(2)
25	S1	27(1)	30(1)	24(1)	4(1)	-2(1)	-5(1)
	O4	31(1)	36(1)	23(1)	9(1)	-1(1)	0(1)
	O5	53(1)	58(1)	37(1)	13(1)	-11(1)	-35(1)
	O6	34(1)	35(1)	28(1)	-3(1)	-2(1)	10(1)
	O7	81(2)	25(1)	40(1)	-1(1)	12(1)	6(1)
30	C21	26(1)	25(2)	24(1)	-1(1)	3(1)	2(1)
	C22	35(2)	25(2)	31(2)	0(1)	1(1)	-1(1)
	C23	40(2)	30(2)	25(1)	-2(1)	1(1)	-2(1)
	C24	28(1)	29(2)	26(2)	2(1)	2(1)	2(1)
	C25	30(2)	34(2)	29(2)	-1(1)	-2(1)	0(1)
35	C26	26(1)	34(2)	28(2)	0(1)	1(1)	-5(1)
	C27	23(1)	26(1)	26(1)	0(1)	2(1)	0(1)
	C28	31(1)	26(1)	30(1)	0(1)	-2(1)	-6(1)
	C29	29(2)	41(2)	40(2)	0(2)	2(1)	-3(1)

40 TABLE E. Hydrogen coordinates [$\times 10^4$] and isotropic displacement parameters [$\text{\AA}^2 \times 10^3$].

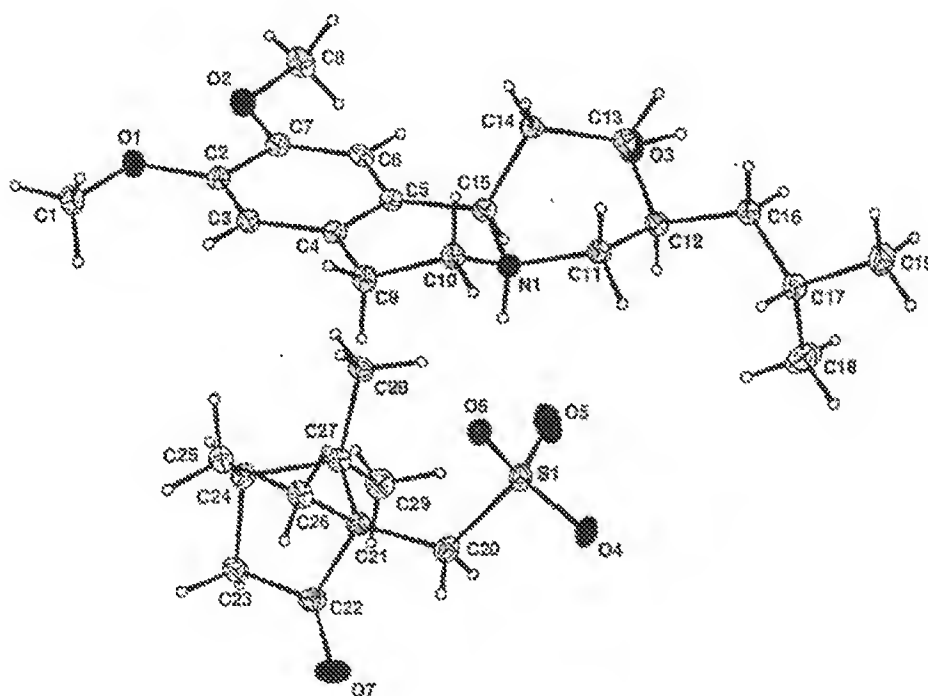
Atom	x	y	z	U_{eq}	S.o.f	
45	H98	5190(40)	10528(15)	2062(10)	70(8)	1
	H99	10030(50)	12950(30)	2575(12)	70(8)	1
	H1A	1107	11933	156	54	1
	H1B	529	12973	-89	54	1

	H1C	-154	12777	395	54	1
	H3	1220	11793	904	30	1
	H6	6760	13337	1353	29	1
	H8A	6872	14966	1009	58	1
5	H8B	7600	15065	523	58	1
	H8C	8193	14091	810	58	1
	H9A	814	11106	1651	33	1
	H9B	2505	10324	1567	33	1
	H10A	2250	11767	2259	32	1
10	H10B	2235	10534	2304	32	1
	H11A	4431	11494	2822	30	1
	H11B	5322	10372	2759	30	1
	H12	8230	11108	2589	30	1
	H13	7334	13145	2840	30	1
15	H14A	4783	13050	2397	30	1
	H14B	6354	13538	2090	30	1
	H15	7056	11776	1864	29	1
	H16A	5973	11796	3278	33	1
	H16B	6813	11911	3386	33	1
20	H17	6493	10098	3412	41	1
	H18A	8906	9588	2944	97	1
	H18B	9176	9031	3400	97	1
	H18C	10440	10005	3276	97	1
	H19A	9329	10894	3971	62	1
25	H19B	8110	9887	4073	62	1
	H19C	7136	10999	4054	62	1
	H20A	8824	7924	1207	37	1
	H20B	6787	7484	1286	37	1
	H23A	6070	8190	-151	38	1
30	H23B	8277	8423	-116	38	1
	H24	6928	10107	-8	33	1
	H25A	3773	9195	153	37	1
	H25B	4152	10235	426	37	1
	H26A	3994	8237	764	35	1
35	H26B	4300	9279	1039	35	1
	H28A	8160	10638	1135	44	1
	H28B	6103	10692	936	44	1
	H28C	7811	11207	684	44	1
	H29A	10358	10042	381	54	1
40	H29B	10159	8817	436	54	1
	H29C	10517	9531	849	54	1

Table 6. Hydrogen bonds [\AA and $^\circ$].

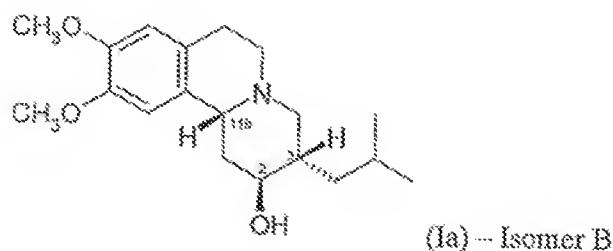
$D-H\cdots A$	$d(D-H)$	$d(H\cdots A)$	$d(D\cdots A)$	$\angle(DHA)$
$N1-H98\cdots O6$	0.885(10)	1.895(12)	2.773(3)	171(3)
$N1-H98\cdots S1$	0.885(10)	2.914(14)	3.771(2)	163(3)
$O3-H99\cdots O4^i$	0.84(4)	1.94(4)	2.766(3)	165(3)
$O3-H99\cdots S1^i$	0.84(4)	2.98(4)	3.811(2)	169(3)

Symmetry transformations used to generate equivalent atoms:

(i) $-x+2, y+1/2, -z+1/2$ 

Thermal ellipsoids drawn at the 30% probability level

On the basis of the data set out above, Isomer B is believed to have the 2S, 3S, 11bR configuration, which corresponds to Formula (Is):



EXAMPLE 5

Sigma Receptor Binding Studies

The four dihydrotetrabenazine isomers A, B, C and D subjected to specific binding assays to test their ability to bind to the sigma-1 and sigma-2 receptors. The results are set out in Table 5.

(a) Sigma σ_1 Receptor:

Reference: Ganapathy *et al.*, *Pharmacol. Exp. Ther.*, 289:251-260, (1999)

Source: Human Jurkat cells

10 Ligand: 8 nM [3 H] Haloperidol

Vehicle: 1% DMSO

Incubation time/Temp: 4 hours @ 25 °C

Incubation buffer: 5 mM K₂HPO₄/KH₂PO₄ buffer pH 7.5

Non Specific ligand: 10 μ M Haloperidol

15 K_d: 5.8 nM

B_{max}: 0.71 pmole/mg protein

Specific binding: 80%

Quantitation method: Radioligand binding

Significance criteria: \geq 50% of maximum stimulation or inhibition

20 (b) Sigma σ_2 Receptor:

Reference: Hashimoto *et al.*, *Eur. J. Pharmacol.*, 236:159-163, (1993)

Source: Wistar rat brain

Ligand: 3 nM [3 H] Ifenprodil

Vehicle: 1% DMSO

25 Incubation time/Temp: 60 minutes @ 37 °C

Incubation buffer: 50 mM Tris-HCl, pH 7.4

Non Specific ligand: 10 μ M Ifenprodil

K_d : 4.8 nM

B_{max} : 1.3 pmole/mg protein

Specific binding: 85%

5 Quantitation method: Radioligand binding

Significance criteria: $\geq 50\%$ of maximum stimulation or inhibition

Table 5

Percentage Inhibition by 10 μ M Solutions of Dihydrotetraabenazine isomers of Specific Binding at Sigma-1 and Sigma-2 Receptors				
Receptor/Protein	Isomer A	Isomer B	Isomer C	Isomer D
(g) σ_1 Receptor	48	82	59	82
(h) σ_2 Receptor	64	64	61	69

The data show that all four isomers bind to both the sigma-1 and sigma-2 receptors.

10 The four isomers are approximately equipotent in the sigma-2 receptor binding studies but isomers B and D show stronger binding to the sigma-1 receptor.

EXAMPLE 6

Anti-proliferative Activity

The anti-proliferative activities of compounds of the invention may be determined by measuring the ability of the compounds to inhibition of cell growth in a number of cell lines. Inhibition of cell growth is measured using the Alamar Blue assay (Nociari, M. M, Shalev, A., Benias, P., Russo, C. *Journal of Immunological Methods* 1998, 213, 157-167). The method is based on the ability of viable cells to reduce resazurin to its fluorescent product resorufin. For each proliferation assay cells are plated onto 96 well plates and allowed to recover for 16 hours prior to the addition of inhibitor compounds for a further 72 hours. At the end of the incubation period 10% (v/v) Alamar Blue is added and incubated for a further 6 hours prior to determination of fluorescent product at 535nm ex / 590nm em. In the case of the non-proliferating cell assay cells are maintained at confluence for 96 hour prior to

the addition of inhibitor compounds for a further 72 hours. The number of viable cells is determined by Alamar Blue assay as before. All cell lines are obtained from ECACC (European Collection of cell Cultures). Examples of such cell lines are the human SK-N-SH neuroblastoma and rat C6 glioma cell lines.

5 EXAMPLE 7

Determination of the effect of compounds of the invention on LPS/IFN stimulated production of cytokines by human monocytes

The compounds of the invention were tested to determine the extent to they can modulate the production of cytokines by human monocytes. Monocytes are
10 precursors of macrophages and are a key source of inflammatory cytokines in a wide range of disease states. The activity of the compounds of the invention against monocyte production therefore provides a good indicator of the likelihood of the compounds possessing anti-inflammatory activity.

The following treatment groups were set up.

Group	Treatment
A	Unstimulated Monocyte Control
F	Unstimulated Monocytes + Nabilone 100µM/10µM/1µM/100nM/10nM/1nM/100pM/10pM
G	Unstimulated Monocytes + Isomer D (100µM/10µM/1µM/100nM/10nM/1nM/100pM/10pM)
H	Unstimulated Monocytes + Isomer B (100µM/10µM/1µM/100nM/10nM/1nM/100pM/10pM)
I	LPS/IFNγ Stimulated Monocytes alone
N	Stimulated Monocytes + Nabilone (100µM/10µM/1µM/100nM/10nM/1nM/100pM/10pM)
O	Stimulated Monocytes + Isomer D (100µM/10µM/1µM/100nM/10nM/1nM/100pM/10pM)
P	Stimulated Monocytes + Isomer B (100µM/10µM/1µM/100nM/10nM/1nM/100pM/10pM)

Procedures

Modulation of human monocyte cytokine production

Materials

MACS Buffer: 1x PBS pH 7.2, 2 mM EDTA (Sigma), 5% human AB serum

- 5 Culture Media: 500 ml RPMI 1640 (Invitrogen), 5 ml Penstrep (Sigma), 5 ml L-glutamine (Invitrogen), 10 ml HEPES (Sigma), 5% autologous plasma.

Compounds: Stock at 10 mM in absolute ethanol.

LPS: Salmonella abortus at 1mg/ml (Sigma, Cat # L1887).

IFN γ : Recombinant human at 10 μ g/ml (BD Pharmacia, Cat # 554617).

- 10 MACS CD14 positive selection beads and LS columns (Milenyi Biotech).
CD14:FITC antibody (Milenyi Biotech)

Buffy coat obtained from Bristol National Blood Services.

1 ml of blood should give 7×10^6 total cells.

Preparation of Autologous Plasma

- 15 Spin buffycoat at 3500 rpm, 10 min.

Remove upper plasma layer and heat-inactivate at 56 °C, 30 min.

Spin at 3500 rpm, 10 min to remove heat-inactivated complement proteins etc.

Peripheral Blood Mononuclear Cell (PBMC) Isolation

1. Resuspend remaining buffycoat in an equal volume of RPMI 1640 medium
20 and mix by inversion.
2. Prewarm histopaque (Sigma) to room temperature (RT) and add 10 ml to a universal.
3. Gently overlay with 15 ml blood/medium mix.
4. Spin at 1600 rpm for 25 minutes at RT (no brake).

5. PBMC are separated into the interface layer between the medium and the histopaque, remove cells to a 50 ml Falcon and add 10 ml medium.

6. Spin at 1800 rpm for 10 min at RT.

7. Wash x3 with 40 ml RPMI medium and resuspend in culture medium.

5 Isolate CD14⁺ cells by positive selection with CD14⁺ MicroBeads

Work on ice, with pre-cooled solutions.

1. Determine cell numbers ($313 \times 10 \times 5 \times 10^4 = 1.565 \times 10^8$ total cells)

2. Remove 100 μ l cell sample for Fluorescence-Activated Cell-Sorting (FACS) analysis (pre-selection sample).

10 3. Spin at 1800 rpm for 10 minutes at 4 °C. Remove supernatant.

4. Resuspend pellet in 80 μ l buffer per 1×10^7 total cells (2400 μ l/total cells).

5. Add 20 μ l CD14 MicroBeads per 1×10^7 total cells (400 μ l/total cells).

6. Mix well and incubate for 15 minutes at 4-8 °C.

15 7. Wash cells with 1-2 ml buffer, spin at $300 \times g$, 10 minutes at 4 °C. Remove supernatant.

8. Resuspend up to 1×10^8 cells in 500 μ l buffer (1 ml/total cells).

Magnetic Separation with LS Column

1. Place column in magnetic field of MACS[®] Separator. Rinse column with 3 ml of MACS buffer.

20 2. Add cell suspension to column.

3. Collect unlabelled cells which pass through. Wash column with 3x 3 ml buffer, allowing the column reservoir to empty between each wash. Collect total effluent (unlabelled cell fraction).

4. Remove column from separator and place on collection tube.

25 5. Add 5 ml buffer to column, immediately flushing out fraction with magnetically labelled cells by applying column plunger.

6. Spin cells in MACS Buffer and resuspend cell pellet in media.

7. Perform cell count on positively selected cells.

$71 \times 20 \times 5 \times 10^4 = 7.1 \times 10^7$ cells/ml. Have 1.75ml $\therefore 1.24 \times 10^8$ total cells.

8. Take sample to check purity by FACS – add 10 µl CD14-FITC antibody and incubate, 5 minutes at 4-8 °C.

Compound Preparation

- Prepare compounds at 10 mM in absolute ethanol. Dilute prior to culture in media to the required concentrations.

Cell Culture

- Isolated CD14⁺ monocytes were cultured in 24-well plates at 1×10^6 cells/ml/well. The monocytes were cultured in the presence of the test compounds and controls at various dilutions as set out in the table below. After overnight incubation, LPS (10 µg/ml) and IFN γ (100 ng/ml) were added to stimulate monocyte activation and elevate CB2 expression. After 48 hours, the supernatants were removed for CBA cytokine analysis.

CBA Cytokine Analysis (BenderMedSystems Human Th1/Th2 Kit [Cat # BMS716FF])

- 15 Bring all buffers to room temperature and vortex before use
1. Make up assay buffer by adding to 450 ml distilled H₂O and mixing gently. Store at 4 °C.
 2. Make up biotin-conjugate mix by 600 µl of each biotin-conjugates into a universal.
 - 20 3. Make up bead mix by adding 300 µl of each bead set.
 4. Reconstitute each standard with distilled H₂O as recommended on vial label. Add 100 µl assay buffer to tube labelled Standard 1. Add 10 µl of each reconstituted standard and mix. Serial dilute standard mixture (100 µl assay buffer to tubes 2-7, add 50 µl standard 1 to tube 2, mix and transfer 50 µl to tube 3 etc).
 - 25 5. Label FACS tubes and add 25 µl of standards 1-7 to designated tubes. Add 25 µl assay buffer to blank tube.
 6. Add 25 µl of sample to designated sample tubes.
 7. Add 25 µl bead mix to all tubes.
 8. Add 50 µl biotin-conjugate to all tubes.
 - 30 9. Mix by vortexing, then cover with foil and incubate for 2 hours @ RT.

10. Prepare Streptavidin-PE solution by mixing 176 μ l in 5324 μ l assay buffer. Add 1 ml assay buffer to all tubes and spin at 200 x g, 5 min. Tip off supernatant. Repeat wash step and tip off supernatant.
11. Add 50 μ l Streptavidin-PE to all tubes. Mix by vortexing, then cover with foil and incubate for 1h @ RT.
12. Repeat wash step x2.
13. Add 500 μ l assay buffer to all tubes.

Flow Cytometer Set-up

- Run positive and negative beads (supplied with kit) to optimise set-up and determine compensation etc. Run standard curve prior to samples, counting 3000 events on gated population (300 events/analyte).

RESULTS

The results are shown in Figures 1 to 6. In the Figures, the label RU348 designates Isomer D.

- As can be seen from Figures 1 to 6, the two *cis*-dihydrotetrabenazine isomers B and D both reduced the production of a range of different cytokines in monocytes that had been stimulated by LPS/IFN.

Thus, Isomer B reduced production of the cytokines IL-2 and IL-12 at all concentrations tested and reduced production of cytokines IL-4, IL-5 and IL-10 at the majority of concentrations tested.

Isomer D reduced production of the cytokines IL-2, IL-4, IL-5 and IL-12 at all concentrations tested and reduced production of cytokines TNF α , IL-4, IL-5 and IL-10 at the majority of concentrations tested.

EXAMPLE 8

- Determination of the effect of compounds of the invention on T Cell Proliferation

The compounds of the invention were tested to determine the extent to they can inhibit T cell proliferation. T cells are responsible for co-ordinating the adaptive immune response and are drivers of inflammation in a wide range of disease states.

The activity of the compounds of the invention against T cell proliferation therefore provides a good indicator of the likelihood of the compounds possessing anti-inflammatory activity.

Materials

- 5 HBSS + 2% HEPES buffer (500 ml+10 ml), Alpha MEM(500 ml) + 2% HEPES(10 ml) + 1% Pen/strep(5 ml) + 2% L-Glutamine(10ml) + 50µM 2-ME(500 µl) and Normal Mouse serum (NMS)

Methods

1. Coat 3 x 96 well plates columns 1-8, rows A-H with 100µl anti-mouse CD3 at 1µg/ml in PBS. 192 well in total, therefore made up 24 mls in PBS by adding 17 µl of stock at 1.44mg/ml. Incubate 2 hours 37 °C.
2. Pooled blood from 2 balb/c mice following cardiac puncture for preparation of NMS and removed spleens into HBSS + Hepes.
3. Placed blood at 37 °C for 30 minutes to clot, spun 3,000 rpm, 10 minutes in 15 chilspin. Removed serum, filter sterilised and kept in fridge until use.
4. Transferred spleens to petri dishes containing 10 ml HBSS, used clean sterile wire mesh placed over spleen to release cells using a glass rod. Washed cells off mesh using pasteur pipette and transferred to a 15ml tube. Spun at 1000 rpm for 10 minutes at room temperature (RT).
- 20 5. Resuspended cells, added 0.5ml sterile DW to lyse RBC, mixed and immediately diluted in HBSS, transfered to 50 ml tube passing through cell strainer (70µM) to remove debris, spun as above, washed cells once more in HBSS and finally resuspended in 2mls alpha MEM containing supplements (as above).
6. Cell counts - $252 \text{ cells} \times 2 \times 5 \times 10^4 = 2.52 \times 10^7 \text{ cells/ml in 4mls} = 10^8 \text{ cells}$ 25 in total. Dilute 2mls to 25mls to give a cell concentration of $2 \times 10^6 \text{ cells/ml}$.
7. Added 250µl NMS to cells to give a concentration of 1%.
8. Washed anti-CD3 coated plates 3 x PBS.
9. Transferred 100µl aliquots of cells to coated wells, rows A-H columns 1-8 of 3 x 96-well plates. Add equal volume of compounds diluted in a 96 well plate as 30 shown below.

Compounds

Transferred 3.5 μ l compound at 10 mM to 346.5 μ l media (1/100), transferred 35 μ l of 1/100 dilution to 315 μ l media (1/10) and so on down plate to give serial 1/10 dilutions of compound. Transferred 100 μ l aliquots of different compound dilutions to wells of triplicate plates containing T cells.

Nab ^a	Isomer D	Isomer B	Media
1/100	100 μ M	100 μ M	100 μ M
1/10	10 μ M	10 μ M	10 μ M
1/10	1 μ M	1 μ M	1 μ M
1/10	100 nM	100 nM	100 nM
1/10	10 nM	10 nM	10 nM
1/10	1 nM	1 nM	1 nM
1/10	100 pM	100 pM	100 pM
1/10	10 pM	10 pM	10 pM

5 * Nab = nabilone

Culture for 48 hours, then add a drop (16 μ l) of ³H-thymidine to each well and incubate overnight at 37 °C 5% CO₂. Harvest. Measure ³H-thymidine incorporation.

Results

10 The extent of T cell proliferation, as demonstrated by levels of ³H-thymidine incorporation, is demonstrated by the data are shown in the Table below.

incorporation, is demonstrated by the data are shown in the Table below.

T cell Proliferation

Plate 1	Nab	RU346	RU350	Media
A	42	37.3	28	36361.9
B	40910.7	94808.1	74.7	42083.8
C	52155.5	55277.3	46179.1	48044.3
D	41607.8	52138	54184.4	53253.6
E	35442	49102.4	53277.2	46372.2
F	37988.7	51285	58024.4	52391.8
G	37202.7	53139.1	46056.8	52016.7
H	38818.1	50802.8	52846.7	64429.5

Plate 2	Nab	RU346	RU350	Media
A	18.7	14	42	68823.2
B	72188.2	108341.6	38743.1	51976.8
C	48534.5	66042.6	62557.5	56244.3
D	65222	48013.1	63867.2	62197.1
E	44970.2	55537.9	66290	42556.2
F	36994.3	50320.9	40054.3	54165.5
G	44820.7	47373.7	60937.4	40598.4
H	34530	52670.8	41897.1	42497

Plate 3	Nab	RU346	RU350	Media
A	46.7	18.7	51.4	63111.1
B	78370.5	84078.6	110594.7	51408.2
C	44235.7	123241.3	58612.8	41777.8
D	55348.5	61529	67792.8	70249.8
E	46765.1	57697.5	70736.7	58283.9
F	35152.2	72051.9	74224	68615.4
G	36358.1	74186.6	59985.8	52697.8
H	39255.7	72608.4	57220.4	42878.1

In the Table, "Nab" refers to Nabilone, "RU346" refers to Isomer D and "RU350" refers to Isomer B.

- 5 The results demonstrate that each of the compounds at the highest concentrations tested inhibit proliferation of T cells.

EXAMPLE 9**Pharmaceutical Compositions****(i) Tablet Formulation - I**

- 10 A tablet composition containing a dihydrotetrabenazine of the invention is prepared by mixing 50mg of the dihydrotetrabenazine with 197mg of lactose (BP) as diluent, and 3mg magnesium stearate as a lubricant and compressing to form a tablet in known manner.

(ii) Tablet Formulation - II

A tablet composition containing a dihydrotetabenazine of the invention is prepared by mixing the compound (25 mg) with iron oxide, lactose, magnesium stearate, starch maize white and talc, and compressing to form a tablet in known manner.

5 (iii) Capsule Formulation

A capsule formulation is prepared by mixing 100mg of a dihydrotetabenazine of the invention with 100mg lactose and filling the resulting mixture into standard opaque hard gelatin capsules.

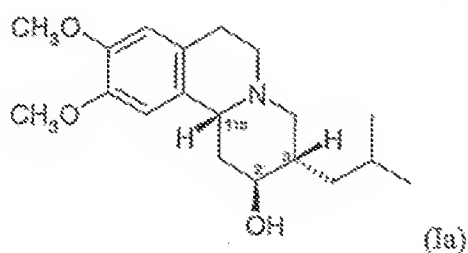
Equivalents

- 10 It will readily be apparent that numerous modifications and alterations may be made to the specific embodiments of the invention described above without departing from the principles underlying the invention. All such modifications and alterations are intended to be embraced by this application.

CLAIMS

1. The use of a compound for the manufacture of a medicament for the prophylaxis or treatment of a proliferative disease or an inflammatory disease, the compound being 3,11b-*cis*-dihydrotetrabenazine or a
5 pharmaceutically acceptable salt thereof.
2. The use according to claim 1 wherein the medicament is for the prophylaxis or treatment of an inflammatory disease.
3. The use according to claim 2 wherein the inflammatory disease is selected from rheumatoid arthritis, osteoarthritis, traumatic arthritis, gouty arthritis,
10 rubella arthritis, psoriatic arthritis, and other arthritic conditions; acute or chronic inflammatory disease states such as the inflammatory reaction induced by endotoxin or inflammatory bowel disease; Reiter's syndrome, gout, rheumatoid spondylitis, chronic pulmonary inflammatory disease, Crohn's disease and ulcerative colitis.
- 15 4. The use according to claim 3 wherein the inflammatory disease is rheumatoid arthritis.
5. A compound for use in the prophylaxis or treatment of an inflammatory disease, for example an inflammatory disease as defined in claim 3 or claim 4, wherein the compound is 3,11b-*cis*-dihydrotetrabenazine
- 20 6. A method for the prophylaxis or treatment of an inflammatory disease or condition (for example an inflammatory disease as defined in claim 3 or claim 4) in a patient (e.g. a mammal such as a human), which method comprises administering to the mammal a therapeutically effective amount of 3,11b-*cis*-dihydrotetrabenazine.
- 25 7. A use, compound for use, or method as defined in any one of claims 1 to 6 wherein the 3,11b-*cis*-dihydrotetrabenazine is Isomer B or Isomer D as defined herein.

8. A use, compound for use, or method as defined in any one of claims 1 to 6 wherein the 3,11b-*cis*-dihydrotetrabenazine has the formula (Ia):



9. A compound for use, use or method as defined in any one of claims 1 to 16
5 wherein the 3,11b-*cis*-dihydrotetrabenazine in the form of an acid addition salt.
10. A use, compound for use, or method as defined in claim 9 wherein the salt is a methane sulphonate salt.
11. A use, compound for use, or method substantially as described herein with
10 reference to the examples.

CBA Figures: All cytokine levels are in pg/ml.

■ Compound + LPS/IFN, ◆ Nabilone + LPS/IFN, ♦ Control (+ EtOH) + LPS/IFN
 □ Compound alone, ◇ Nabilone alone, ◊ Control (+ EtOH)

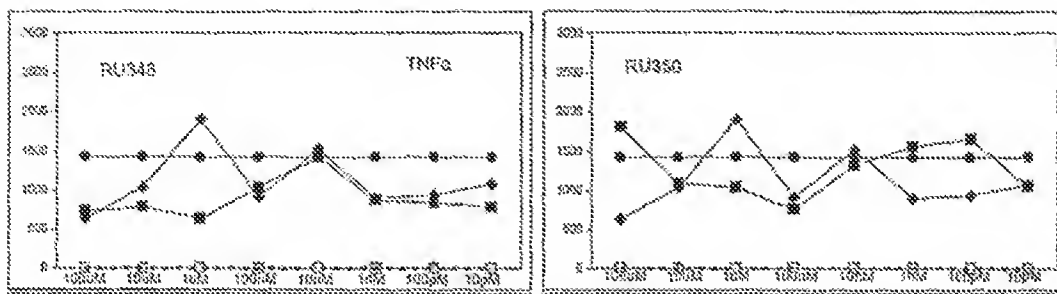


Figure 1

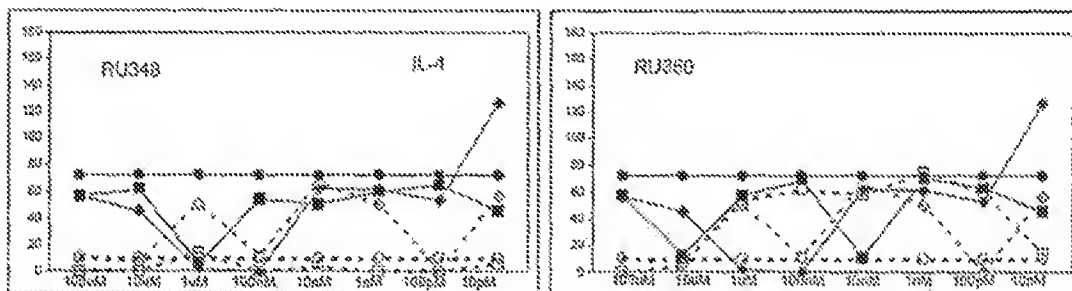


Figure 2

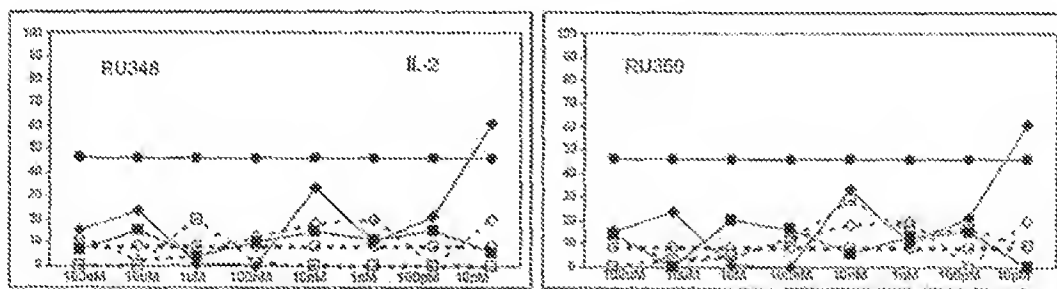


Figure 3

2/2

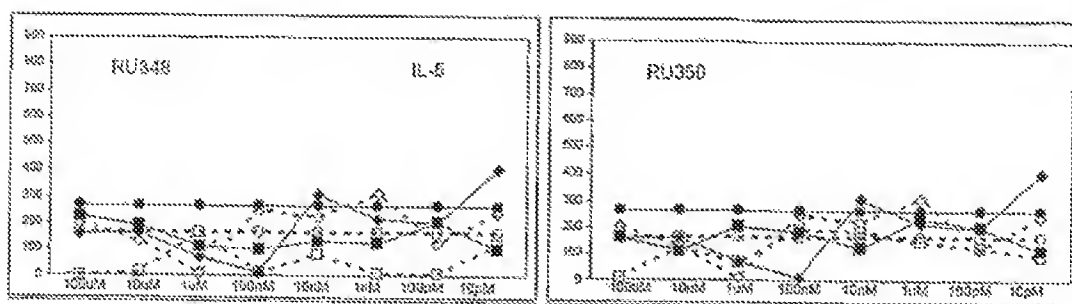


Figure 4

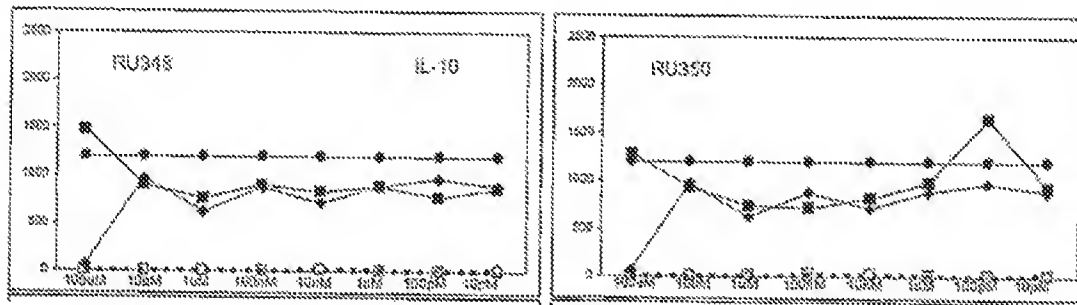


Figure 5

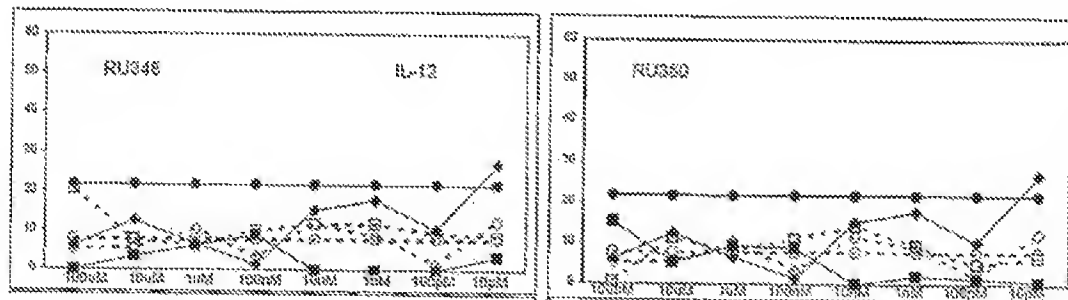


Figure 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB2006/002909

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/473 A61P19/02 A61P29/00 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data bases consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MEHVAR R ET AL: "CONCENTRATION-EFFECT RELATIONSHIPS OF TETRABENAZINE AND DIHYDROTETRABENAZINE IN THE RAT" JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 76, no. 6, 1987, pages 461-465, XP009075267 ISSN: 0022-3549 cited in the application last paragraph	I-11

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"I" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (see specification)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

Date of the actual completion of the international search

12 December 2006

Date of mailing of the international search report

27/12/2006

Name and mailing address of the ISA/

European Patent Office, P. B. 5816 Patentlaan 2
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Büttner, Ulf

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB2006/002909

(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCHAEFER A ET AL: "Effects of biogenic amines and psychotropic drugs on endogenous prostaglandin biosynthesis in the rat brain homogenates" BIOCHEMICAL PHARMACOLOGY 1978 UNITED KINGDOM, vol. 27, no. 2, 1978, pages 213-218, XP009075242 table 1	1-11
P, X	WO 2005/077946 A (CAMBRIDGE LAB LTD [GB]; TRIDGETT ROBERT [GB]; CLARKE IAN [GB]; TURTLE) 25 August 2005 (2005-08-25) the whole document	5, 8, 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2006/002909

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 5, 7, 13, 15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 5.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2006/002909

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005077946 A	25-08-2005	AU 2005213525 A1	25-08-2005
		CA 2555815 A1	25-08-2005
		EP 1716145 A1	02-11-2006